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<b>(54) Title:</b> SUBSTITUTED DI- AND TRIPEPTIDE INHIBITORS OF PROTEIN:FARNESYL TRANSFERASE			
<b>(57) Abstract</b>  Novel inhibitors of protein:farnesyl transferase enzyme are described, as well as methods for the preparation and pharmaceutical compositions of the same, which are useful in controlling tissue proliferative diseases, including cancer and restenosis.			

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SUBSTITUTED DI- AND TRIPEPTIDE INHIBITORS  
OF PROTEIN:FARNESYL TRANSFERASE

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## FIELD OF THE INVENTION

The present invention pertains to a number of compounds which can be used in the medicinal field to treat, prophylactically or otherwise, uncontrolled or abnormal proliferation of human tissues. More specifically, the present invention pertains to a number of compounds which act to inhibit the farnesyl transferase enzyme that has been determined to activate ras proteins which in turn activate cellular division and are implicated in cancer and restenosis.

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## BACKGROUND OF THE INVENTION

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Ras protein (or p21) has been examined extensively because mutant forms are found in 20% of most types of human cancer and greater than 50% of colon and pancreatic carcinomas (Gibbs J.B., Cell, 65:1 (1991), Cartwright T., et al., Chimica Oggi, 10:26 (1992)).

25

These mutant ras proteins are deficient in the capability for feedback regulation that is present in native ras and this deficiency is associated with their oncogenic action since the ability to stimulate normal cell division can not be controlled by the normal endogenous regulatory cofactors. The recent discovery that the transforming activity of mutant ras is critically dependent on post-translational modifications (Gibbs J., et al., Microbiol. Rev., 53:171 (1989)) has unveiled an important aspect of ras function and identified novel prospects for cancer therapy.

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In addition to cancer, there are other conditions of uncontrolled cellular proliferation that may be related to excessive expression and/or function of native ras proteins. Post surgical vascular restenosis is such a condition. The use of various surgical revascularization techniques such as saphenous vein bypass grafting, endarterectomy and transluminal coronary angioplasty is often accompanied by complications due to uncontrolled growth of neointimal tissue, known as restenosis. The biochemical causes of restenosis are poorly understood and numerous growth factors and protooncogenes have been implicated (Naftilan A.J., et al., Hypertension, 13:706 (1989) and J. Clin. Invest., 83:1419; Gibbons G.H., et al., Hypertension, 14:358 (1989); Satoh T., et al., Mollec. Cell. Biol., 13:3706 (1993)). The fact that ras proteins are known to be involved in cell division processes makes them a candidate for intervention in many situations where cells are dividing uncontrollably. In direct analogy to the inhibition of mutant ras related cancer, blockade of ras dependant processes has the potential to reduce or eliminate the inappropriate tissue proliferation associated with restenosis, particularly in those instances where normal ras expression and/or function is exaggerated by growth stimulatory factors.

Ras functioning is dependent upon the modification of the proteins in order to associate with the inner face of plasma membranes. Unlike other membrane-associated proteins, ras proteins lack conventional transmembrane or hydrophobic sequences and are initially synthesized in a cytosol soluble form. Ras protein membrane association is triggered by a series of posttranslational processing steps that are signaled by a carboxyl terminal amino acid consensus sequence that is recognized by protein:farnesyl transferase.

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This consensus sequence consists of a cysteine residue located four amino acids from the carboxyl terminus, followed by two lipophilic amino acids and the C-terminal residue. The sulfhydryl group of the cysteine residue is alkylated by farnesyl pyrophosphate in a reaction that is catalyzed by protein:farnesyl transferase. Following prenylation, the C-terminal three amino acids are cleaved by an endoprotease and the newly exposed alpha-carboxyl group of the prenylated cysteine is methylated by a methyl transferase. The enzymatic processing of ras proteins that begins with farnesylation enables the protein to associate with the cell membrane. Mutational analysis of oncogenic ras proteins indicate that these posttranslational modifications are essential for transforming activity. Replacement of the consensus sequence cysteine residue with other amino acids gives a ras protein that is no longer farnesylated, fails to migrate to the cell membrane and lacks the ability to stimulate cell proliferation (Hancock J.F., et al., Cell, 57:1617 (1989); Schafer W.R., et al., Science, 245:379 (1989); Casey P.J., Proc. Natl. Acad. Sci. USA, 86:8323 (1989)).

Recently, protein:farnesyl transferases (PFTs, also referred to as farnesyl:protein transferases) have been identified and a specific PFT from rat brain was purified to homogeneity (Reiss Y., et al., Bioch. Soc. Trans., 20:487-88 (1992)). The enzyme was characterized as a heterodimer composed of one alpha-subunit (49 kDa) and one beta-subunit (46 kDa), both of which are required for catalytic activity. High level expression of mammalian PFT in a baculovirus system and purification of the recombinant enzyme in active form has also been accomplished (Chen W.-J., et al., J. Biol. Chem., 268:9675 (1993)).

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In light of the foregoing, the discovery that the function of oncogenic ras proteins is critically dependent on their posttranslational processing provides a means of cancer chemotherapy through inhibition of the processing enzymes. The identification and isolation of a protein:farnesyl transferase that catalyzes the addition of a farnesyl group to ras proteins provides a promising target for such intervention. Recently it has been determined that prototypical inhibitors of PFT can inhibit ras processing and reverse cancerous morphology in tumor cell models (Kohl N.E., et al., Science, 260:1934 (1993); James G.L., et al., Science, 260:1937 (1993); Garcia A.M., et al., J. Biol. Chem., 268:18415 (1993)). Thus, it is possible to prevent or delay the onset of cellular proliferation in cancers that exhibit mutant ras proteins by blocking PFT. By analogous logic, inhibition of PFT would provide a potential means for controlling cellular proliferation associated with restenosis, especially in those cases wherein the expression and/or function of native ras is overstimulated.

PCT Application WO91/16340 discloses cysteine containing tetrapeptide inhibitors of PFT of the formula CAAX.

European Patent Application 0461869 discloses cysteine containing tetrapeptide inhibitors of PFT of the formula Cys-Aaa<sup>1</sup>-Aaa<sup>2</sup>-Xaa.

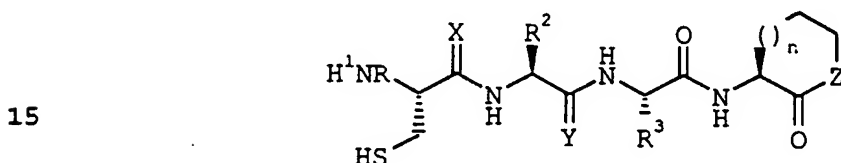
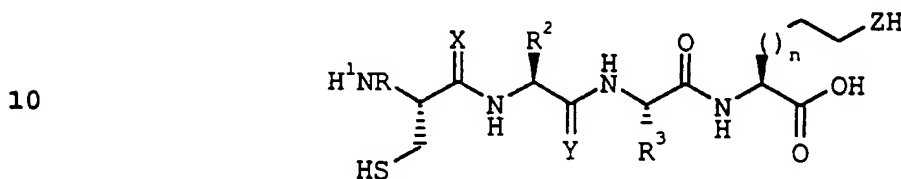
European Patent Application 0520823 discloses cysteine containing tetrapeptide inhibitors of PFT of the formula Cys-Xaa<sup>1</sup>-dXaa<sup>2</sup>-Xaa<sup>3</sup>.

European Patent Application 0523873 discloses cysteine containing tetrapeptide inhibitors of PFT of the formula Cys-Xaa<sup>1</sup>-Xaa<sup>2</sup>-Xaa<sup>3</sup>.

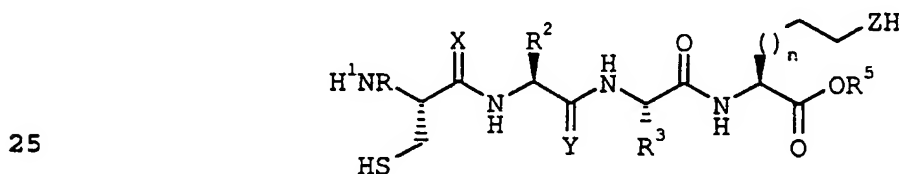
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European Patent Application 0528486 discloses cysteine containing tetrapeptide amides inhibitors of PFT of the formula Cys-Xaa<sup>1</sup>-Xaa<sup>2</sup>-Xaa<sup>3</sup>-NRR<sup>1</sup>.

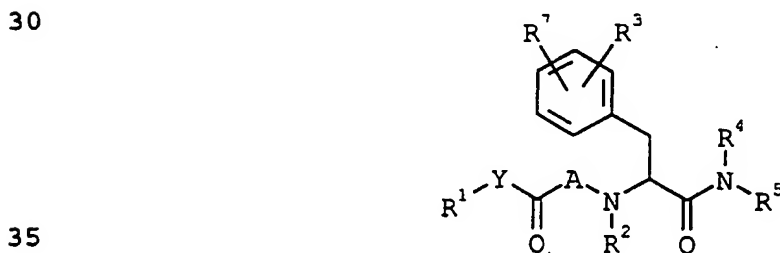
European Patent Application 0535730 discloses pseudotetrapeptide inhibitors of PFT of the following two formulas:



European Patent Application 0535731 (US 5,238,922) discloses esters of pseudotetrapeptide inhibitors of PFT of the formula:



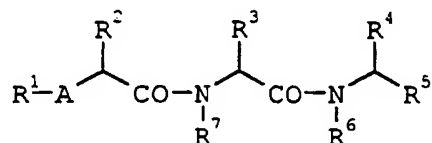
European Patent Application 0482539 discloses tachykinin antagonists of the formula:



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European Patent Application 0457195 discloses  
endothelin antagonists of the formula:

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US 4,022,759 discloses tripeptide antagonists of  
luteinizing hormone releasing factor of the formula  
A-R<sub>1</sub>-Tyr(benzyl)-Ser(benzyl)-R<sub>2</sub>, wherein one of the  
definitions of R<sub>1</sub> is His(benzyl).

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Compounds disclosed in the above references do not  
disclose or suggest a novel combination of structural  
variations found in the present invention described  
hereinafter. All cited references are hereby  
incorporated by reference.

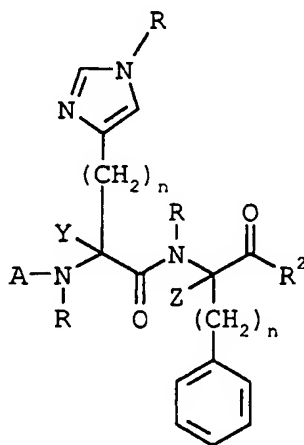
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## SUMMARY OF THE INVENTION

Accordingly, the present invention is a  
substituted di- or tripeptide compound of Formula I:

25

30



35

I



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wherein:

n = 1 or 2;

A = COR<sup>3</sup>, CO<sub>2</sub>R<sup>3</sup>, CONHR<sup>3</sup>, CSR<sup>3</sup>, C(S)OR<sup>3</sup>, C(S)NHR<sup>3</sup>,  
 CF<sub>3</sub>SO<sub>2</sub>, aryl-SO<sub>2</sub>, or alkyl-SO<sub>2</sub>, wherein R<sup>3</sup> is  
 5 alkyl, (CH<sub>2</sub>)<sub>m</sub>-cycloalkyl, (CH<sub>2</sub>)<sub>m</sub>-aryl,  
 (CH<sub>2</sub>)<sub>m</sub>-heteroaryl, or (CH<sub>2</sub>)<sub>m</sub>O-alkyl, and m = 0, 1,  
 2, or 3;

R = independently H or Me;

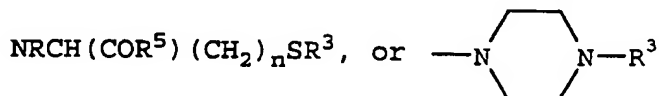
Y = independently H or Me;

10 Z = independently H or Me;

R<sup>1</sup> = H, CO-aryl, (CH<sub>2</sub>)<sub>m</sub>-aryl, O(CH<sub>2</sub>)<sub>m</sub>-cycloalkyl,  
 O(CH<sub>2</sub>)<sub>m</sub>-aryl, or O(CH<sub>2</sub>)<sub>m</sub>-heteroaryl, wherein m is  
 as defined above and R<sup>1</sup> is located at either the  
 meta or para position;

15 X = one to four substituents, including H, alkyl, CF<sub>3</sub>,  
 F, Cl, Br, I, HO, MeO, NO<sub>2</sub>, NH<sub>2</sub>, N(Me)<sub>2</sub>, OPO<sub>3</sub>H<sub>2</sub>, or  
 CH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub>;

R<sup>2</sup> = NR(CH<sub>2</sub>)<sub>n</sub>CO<sub>2</sub>R<sup>3</sup>, NR(CH<sub>2</sub>)<sub>n</sub>CONHR<sup>3</sup>, NR(CH<sub>2</sub>)<sub>n</sub>R<sup>3</sup>,  
 NR(CH<sub>2</sub>)<sub>n+1</sub>OR<sup>4</sup>, NR(CH<sub>2</sub>)<sub>n+1</sub>SR<sup>4</sup>,  
 20 NRCH(COR<sup>5</sup>)(CH<sub>2</sub>)<sub>n</sub>-heteroaryl, NRCH(COR<sup>5</sup>)(CH<sub>2</sub>)<sub>n</sub>OR<sup>3</sup>,



25 wherein R, R<sup>3</sup>, and n are as defined above, R<sup>4</sup> = H  
 or R<sup>3</sup>, and R<sup>5</sup> = OH, NH<sub>2</sub>, OR<sup>3</sup>, or NHR<sub>3</sub>; an optical  
 isomer, diastereomer, or a pharmaceutically  
 acceptable salt thereof.

The present invention is also directed to the use  
 of a compound of Formula I, or a pharmaceutically  
 30 acceptable salt therefrom, to inhibit the activity of a  
 protein:farnesyl transferase enzyme as a method for  
 treating tissue proliferative diseases.

A further embodiment of the present invention is  
 the use of a pharmaceutical composition including an  
 35 effective amount of a compound of Formula I as a method  
 for the treatment of cancer.

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A still further embodiment of the present invention is the use of a pharmaceutical composition including an effective amount of a compound of Formula I as a method for the treatment of restenosis.

5 A still further embodiment of the present invention is a pharmaceutical composition for administering an effective amount of a compound of Formula I in unit dosage form in the treatment methods mentioned above.

10 A final embodiment of the present invention pertains to methods for the preparation of compounds of Formula I by solid phase synthesis, solution phase synthesis, and simultaneous multiple syntheses using a multiple simultaneous synthesis apparatus.

15

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

20 In the compounds of Formula I, the term "alkyl" means a straight or branched hydrocarbon radical having from 1 to 6 carbon atoms and includes, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl, tert-butyl, n-pentyl, n-hexyl, and the like.

25 The term "cycloalkyl" means a saturated hydrocarbon ring which contains from 3 to 10 carbon atoms, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, adamantyl, and the like, unsubstituted or substituted by an alkyl or aryl group.

30 The term "aryl" means an aromatic ring which is a phenyl, 5-fluorenyl, 1-naphthyl or 2-naphthyl group, unsubstituted or substituted by 1 to 3 substituents selected from alkyl, O-alkyl and S-alkyl, O-aryl, OH, SH, F, Cl, Br, I, CF<sub>3</sub>, NO<sub>2</sub>, NH<sub>2</sub>, NHCH<sub>3</sub>, N(CH<sub>3</sub>)<sub>2</sub>, NHCO-alkyl, (CH<sub>2</sub>)<sub>m</sub>CO<sub>2</sub>H, (CH<sub>2</sub>)<sub>m</sub>CO<sub>2</sub>-alkyl, (CH<sub>2</sub>)<sub>m</sub>SO<sub>3</sub>H, 35 (CH<sub>2</sub>)<sub>m</sub>PO<sub>3</sub>H<sub>2</sub>, (CH<sub>2</sub>)<sub>m</sub>PO<sub>3</sub>(alkyl)<sub>2</sub>, (CH<sub>2</sub>)<sub>m</sub>SO<sub>2</sub>NH<sub>2</sub>, and

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(CH<sub>2</sub>)<sub>m</sub>SO<sub>2</sub>NH-alkyl wherein alkyl is defined as above and m = 0, 1, 2, or 3.

The term "heteroaryl" means a heteroaromatic ring which is a 2- or 3-thienyl, 2- or 3-furanyl, 2- or 3-pyrrolyl, 2-, 3- or 4-pyridyl, 2-, 3-, 4-, 5-, 6-, or 7-indolyl group, unsubstituted or with 1 or 2 substituents from the group of substituents described above for aryl.

The following table provides a list of abbreviations and definitions thereof used in the present invention.

#### TABLE OF ABBREVIATIONS

15	<u>Abbreviation*</u>	<u>Amino Acid</u>
	Ala	Alanine
	Arg	Arginine
	Asn	Asparagine
	Asp	Aspartic acid
20	Cys	Cysteine
	Glu	Glutamic acid
	Gln	Glutamine
	Gly	Glycine
	His	Histidine
25	Ile	Isoleucine
	Leu	Leucine
	Lys	Lysine
	Met	Methionine
	Phe	Phenylalanine
30	Pro	Proline
	Ser	Serine
	Thr	Threonine
	Trp	Tryptophan

\* If the optical activity of the amino acid is other than L(S), the amino acid or abbreviation is preceded by the appropriate configuration D(R) or DL(RS).

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	<u>Abbreviation*</u>	<u>Amino Acid</u> (continued)
	Tyr	Tyrosine
	Val	Valine
5	<u>Abbreviation*</u>	<u>Modified and Unusual Amino Acid</u>
	Aaa-CO <sub>2</sub> R	An amino acid ester, for examples: Gly-CO <sub>2</sub> Bn is Glycine, benzyl ester; Ser(OBn)-CO <sub>2</sub> Me is O-Benzyl-serine, methyl ester.
10	Aaa-CONHR	An amino acid amide, for examples: Gly-CONHBn is Glycine, N-benzyl amide; Ser(OBn)-CONHEt is O-Benzyl-serine, N-ethyl amide; Tyr(OBn)-CONHCH <sub>2</sub> CH <sub>2</sub> OBn is O-Benzyl- tyrosine, N-(2-(phenylmethoxy)ethyl) amide.
15	3Hyp	3-Hydroxyproline
	4Hyp	4-Hydroxyproline
	Hcy	Homocysteine
20	Nva	Norvaline
	Nle	Norleucine
	Orn	Ornithine
	Bal	Beta-alanine (or 3-aminopropionic acid)
25	Abu	4-Aminobutyric acid
	Ahe	7-Aminoheptanoic acid
	Acp	6-Aminocaproic acid
	Aoc	8-Aminooctanoic acid
	Apn	5-Aminopentanoic acid
30	Bpa	(4-Benzoylphenyl)alanine
	Chx	3-Cyclohexylalanine (or Hexahydrophenylalanine)
	Cit	Citrulline

35 \* If the optical activity of the amino acid is other than L(S),  
the amino acid or abbreviation is preceded by the appropriate  
configuration D(R) or DL(RS).

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<u>Abbreviation*</u>	<u>Modified and Unusual Amino Acid</u> (continued)
His(1-Me)	1-Methyl-histidine (or N( $\tau$ )-Methyl-histidine)
5 His(Tr)	1-Triphenylmethyl-histidine (or N( $\tau$ )-Trityl-histidine)
homOPhe	2-Amino-4-phenylbutanoic acid (or Homophenylalanine)
10 homoTyr	2-Amino-4-(4-hydroxyphenyl)butanoic acid (or Homotyrosine)
homoTyr(OBn)	2-Amino-4-[4-(phenylmethoxy)phenyl]-butanoic acid (or O-Benzyl-homotyrosine)
1-Nal	3-(1'-Naphthyl)alanine
15 2-Nal	3-(2'-Naphthyl)alanine
Pen	Penicillamine
Phe(3-OBn)	(3-Benzoyloxyphenyl)alanine
Phe(4-Ph)	3-(1,1'-Biphen-4-yl)alanine (or 4-Phenyl-phenylalanine)
20 Pgl	Phenylglycine
Pyr	2-Amino-3-(3-pyridyl)-propanoic acid (or 3-Pyridylalanine)
Ser(OBn)	O-Benzyl-serine
Thr(OBn)	O-Benzyl-threonine
25 Tic	1,2,3,4-Tetrahydro-3-isoquinoline-carboxylic acid
Tyr(OMe)	O-Methyl-tyrosine
Tyr(OEt)	O-Ethyl-tyrosine
Tyr(OBn)	O-Benzyl-tyrosine
30 ( $\alpha$ -Me)Tyr(OBn)	2-Amino-3-(4-benzyloxyphenyl)-2-methyl-propionic acid (or $\alpha$ -Methyl-O-benzyl-tyrosine)
(N-Me)Tyr(OBn)	N-Methyl-O-benzyl-tyrosine
35 Trp(For)	N <sup>in</sup> -Formyltryptophan

\* If the optical activity of the amino acid is other than L(S), the amino acid or abbreviation is preceded by the appropriate configuration D(R) or DL(RS).

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	<u>Abbreviation</u>	<u>Mercapto Acids</u>
	Maa	Mercaptoacetic acid
	Mba	4-Mercaptobutyric acid
	Mpa	3-Mercaptopropionic acid
5		
	<u>Abbreviation</u>	<u>Protecting Group</u>
	Ac	Acetyl
	Ada	1-Adamantyl acetic acid
	Adoc	Adamantyloxycarbonyl
10	Bn	Benzyl
	MeBn	4-Methylbenzyl
	Cbz	Benzyloxycarbonyl
	2-Br-Cbz	ortho-Bromobenzyloxycarbonyl
	2-Cl-Cbz	ortho-Chlorobenzyloxycarbonyl
15	Bom	Benzyloxymethyl
	Boc	tertiary Butyloxycarbonyl
	Dnp	2,4-Dinitrophenyl
	For	Formyl
	Fmoc	9-Fluorenylmethyloxycarbonyl
20	NO <sub>2</sub>	Nitro
	TMS	Trimethylsilyl
	Tos	4-Toluenesulfonyl (tosyl)
	Tr	Triphenylmethyl (trityl)
25	<u>Abbreviation</u>	<u>Solvents and Reagents</u>
	HOAc	Acetic acid
	CF <sub>3</sub> SO <sub>2</sub> H	Trifluoromethanesulfonic acid
	DCM	Dichloromethane
	DCC	N,N'-Dicyclohexylcarbodiimide
30	DIC	N,N'-Diisopropylcarbodiimide
	DIEA	N,N-Diisopropylethylamine
	DMAP	4-Dimethylaminopyridine
	DMF	N,N'-Dimethylformamide
	EDAC	N-Ethyl-N'-Dimethylaminopropyl-
35		carbodiimide
	EtOAc	Ethyl acetate

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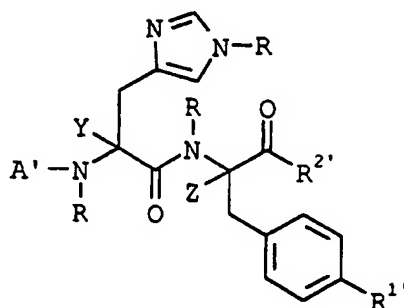
	<u>Abbreviation</u>	<u>Solvents and Reagents</u>
	Et <sub>2</sub> O	Diethyl ether
	HCl	Hydrochloric acid
	HF	Hydrofluoric acid
5	HOBT	1-Hydroxybenzotriazole
	KOH	Potassium hydroxide
	MeCN	Acetonitrile
	MeOH	Methanol
	NHOS	N-Hydroxysuccinimide
10	NMP	N-Methylpyrrolidone
	iPrOH	iso-Propanol
	TBAF	Tetra n-Butylammonium Fluoride
	TFA	Trifluoroacetic acid
15	<u>Abbreviation</u>	<u>Solid Phase Peptide Synthesis Resins</u>
	HMP Resin	4-(Hydroxymethyl)-phenoxymethyl-polystyrene resin
	MBHA Resin	Methylbenzhydrylamine resin
	PAM Resin	4-(Hydroxymethyl)-phenylacetamidomethyl-polystyrene resin
20		
	2-Cl-Tr Resin	2-Chlorotrityl-polystyrene resin
	NH <sub>2</sub> -Rink Resin	4-(amino-(2',4'-dimethoxy-phenyl)methyl)-phenoxymethyl-polystyrene resin
25		
	<u>Abbreviation</u>	<u>Biological Reagents</u>
	FPP	Farnesyl pyrophosphate
	PFT	Protein:farnesyl transferase
30	DTT	Dithiothreitol
	BSA	Bovine serum albumin





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Preferred compounds of Formula I consist of compounds of Formula II below:



II

wherein:

A' = CO<sub>2</sub>R<sup>3</sup>, CONHR<sup>3</sup>, C(S)NHR<sup>3</sup>, or aryl-SO<sub>2</sub>, wherein R<sup>3</sup> is alkyl, (CH<sub>2</sub>)<sub>m</sub>-cycloalkyl, (CH<sub>2</sub>)<sub>m</sub>-aryl, (CH<sub>2</sub>)<sub>m</sub>-heteroaryl, and m = 0, 1, 2, or 3;

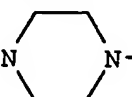
R = independently H or Me;

Y = independently H or Me;

Z = independently H or Me;

R<sup>1'</sup> = (CH<sub>2</sub>)<sub>m</sub>-aryl, O(CH<sub>2</sub>)<sub>m</sub>-aryl, OPO<sub>3</sub>H<sub>2</sub>, or CH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub>, wherein m is as defined above;

R<sup>2'</sup> = NR(CH<sub>2</sub>)<sub>2</sub>OR<sup>4</sup>, NR(CH<sub>2</sub>)<sub>2</sub>SR<sup>4</sup>, NRCH(COR<sup>5</sup>)CH<sub>2</sub>OR<sup>3</sup>,

NRCH(COR<sup>5</sup>)CH<sub>2</sub>SR<sup>3</sup>, or ,  N-CH<sub>2</sub>Ph

wherein R, R<sup>3</sup>, and n are as defined above, R<sup>4</sup> = H or R<sup>3</sup>, and R<sup>5</sup> = OH, NH<sub>2</sub>, OR<sup>3</sup>, or NHR<sup>3</sup>; an optical isomer, diastereomer, or a pharmaceutically acceptable salt thereof.

Other preferred compounds of the present invention are those of Formula I as defined above wherein A is CO<sub>2</sub>R<sup>3</sup> or CONHR<sup>3</sup>; or as defined above in Formula I wherein at least one of Y and Z is Me; or as defined above in Formula I wherein R<sup>2</sup> is (CH<sub>2</sub>)<sub>2</sub>OR<sup>4</sup> or CH(COR<sup>5</sup>)CH<sub>2</sub>OR<sup>3</sup>; or as defined above in Formula I

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wherein A is  $\text{CONHR}^3$ ,  $\text{R}^2$  is  $(\text{CH}_2)_2\text{OR}^4$ , and at least one of Y and Z is Me.

The most preferred compounds of Formula I include the following:

- 5        Cbz-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;
- Cbz-His-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;
- Cbz-His-Tyr(OBn)-Ser(OBn)-CONHEt;
- Cbz-His-Tyr(OBn)-Ser(OBn);
- Cbz-His-Tyr(OBn)-D-Ser(OBn)-CO<sub>2</sub>Me;
- 10       Cbz-D-His-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;
- Cbz-D-His-Tyr(OBn)-Ser(OBn)-CONHEt;
- Cbz-D-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;
- Cbz-D-His-Tyr(OBn)-Ser(OBn);
- Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;
- 15       Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;
- Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn)-CONHEt;
- Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn);
- Cbz-D-His(1-Me)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;
- Cbz-D-His(1-Me)-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;
- 20       Cbz-D-His(1-Me)-Tyr(OBn)-Ser(OBn)-CONHEt;
- Cbz-D-His(1-Me)-Tyr(OBn)-Ser(OBn);
- Cbz-His-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;
- Cbz-His-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;
- Cbz-His-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn)-CONHEt;
- 25       Cbz-His-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn);
- Cbz-His-D-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;
- Cbz-His-D-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;
- Cbz-His-D-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn)-CONHEt;
- Cbz-His-D-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn);
- 30       Cbz-D-His-homoTyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;
- Cbz-His-Phe(4-Ph)-Ser(OBn)-CO<sub>2</sub>Me;
- Cbz-D-His-Phe(4-Ph)-Ser(OBn)-CO<sub>2</sub>Me;
- Cbz-His-Tyr(OBn)-Pyr-CO<sub>2</sub>Me;
- Cbz-D-His-Tyr(OBn)-Pyr-CO<sub>2</sub>Me;
- 35       Cbz-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>OBn;
- Cbz-D-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>OBn;

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- Cbz-His- (N-Me) Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>OBn;  
 Cbz-D-His- (N-Me) Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>OBn;  
 Cbz-His-Tyr (OBn) - CONH(CH<sub>2</sub>)<sub>2</sub>Ph;  
 Cbz-D-His-Tyr (OBn) - CONH(CH<sub>2</sub>)<sub>2</sub>Ph;  
 5 Cbz-His-Tyr (OBn) - Gly-CO<sub>2</sub>Bn;  
 Cbz-D-His-Tyr (OBn) - Gly-CO<sub>2</sub>Bn;  
 Cbz-His-Tyr (OBn) - Gly-CONHBn;  
 Cbz-D-His-Tyr (OBn) - Gly-CONHBn;  
 BnNHCO-His-Tyr (OBn) - Ser (OBn) - CO<sub>2</sub>Me;  
 10 BnNHCO-His-Tyr (OBn) - Ser (OBn) - CONH<sub>2</sub>;  
 BnNHCO-His-Tyr (OBn) - Ser (OBn) - CONHET;   
 BnNHCO-His-Tyr (OBn) - Ser (OBn) ;  
 BnNHCO-His-Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>OBn;  
 BnNHCO-His-Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OPh;  
 15 BnNHCO-D-His-Tyr (OBn) - Ser (OBn) - CO<sub>2</sub>Me;  
 BnNHCO-D-His-Tyr (OBn) - Ser (OBn) - CONH<sub>2</sub>;  
 BnNHCO-D-His-Tyr (OBn) - Ser (OBn) - CONHET;  
 BnNHCO-D-His-Tyr (OBn) - Ser (OBn) ;  
 BnNHCO-D-His-Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>OBn;  
 20 BnNHCO-D-His-Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OPh;  
 Cbz-His-Tyr (OBn) - CON (Me) CH<sub>2</sub>CH<sub>2</sub>OBn;  
 (4-EtOPh)NHCO-D-His-Tyr (OBn) - CONH(CH<sub>2</sub>)<sub>3</sub>OPh;  
 PhCH<sub>2</sub>CO-D-His-Tyr (OBn) - CONH(CH<sub>2</sub>)<sub>3</sub> - (2-MeOPh) ;  
 (4-PhOPh)NHCO-D-His-Tyr (OBn) - COHN(CH<sub>2</sub>)<sub>2</sub>Ph; and  
 25 (4-MePh)SO<sub>2</sub>-D-His-Tyr (OBn) - CO (4-Bn-  
 piperazin-1-yl) .

30 GENERAL METHODS FOR THE PREPARATION, EVALUATION  
 AND USE OF COMPOUNDS OF FORMULA I

The compounds of Formula I may be prepared by  
 solid phase peptide synthesis on a peptide synthesizer,  
 for example, an Applied Biosystems 431A peptide  
 35 synthesizer using activated esters or anhydrides of Boc  
 or Fmoc protected amino acids, acid chlorides,

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isocyanates, isothiocyanates, etc., on PAM, MBHA, or NH<sub>2</sub>-Rink resins with solution phase modifications to the carboxyl terminus as appropriate. Methodology for the solid phase synthesis of peptides is widely known to those skilled in the art thereof (see, for example: J.M. Stewart and J.D. Young in Solid Phase Peptide Synthesis; Pierce Chemical Co.; Rockford, IL (1984); Fields G.B. and Noble R.L., Int. J. Peptide Protein Res., 35:161-214 (1990)).

Additionally, the compounds of Formula I may also be prepared by conventional solution peptide synthesis, substituting amines, acid chlorides, isocyanates, etc, for amino acid derivatives where appropriate. Methods for solution phase synthesis of peptides are widely known to those skilled in the art (see, for example, M. Bodanszky, Principles of Peptide Synthesis, Springer-Verlag (1984)).

Finally, the compounds of Formula I may be prepared by simultaneous multiple solid phase syntheses using an apparatus described by S. H. DeWitt, et al., Proc. Natl. Acad. Sci. USA, 90:6909 (1993), and referred to by the trademark, Diversomer™, both trademark and apparatus being owned in whole by the Warner-Lambert Company. The multiple solid phase synthesis apparatus is currently the subject of now abandoned US Serial 07/958,383 filed October 8, 1992 and pending continuation-in-part US Serial 08/012,557 filed February 2, 1993.

For example (Scheme I below), Fmoc-D-His-Tyr(OBn)-CO<sub>2</sub>-CH<sub>2</sub>CH<sub>2</sub>Si(CH<sub>3</sub>)<sub>3</sub> is linked to 2-Cl-Tr resin using a sterically hindered amine such as DIEA as an HCl scavenger, the Fmoc protecting group is removed with piperidine, the resulting free amino terminus is acylated with a series of isocyanates, isothiocyanates, activated esters, acid chlorides and the like, the TMS-ethyl ester is cleaved with TBAF, the resulting

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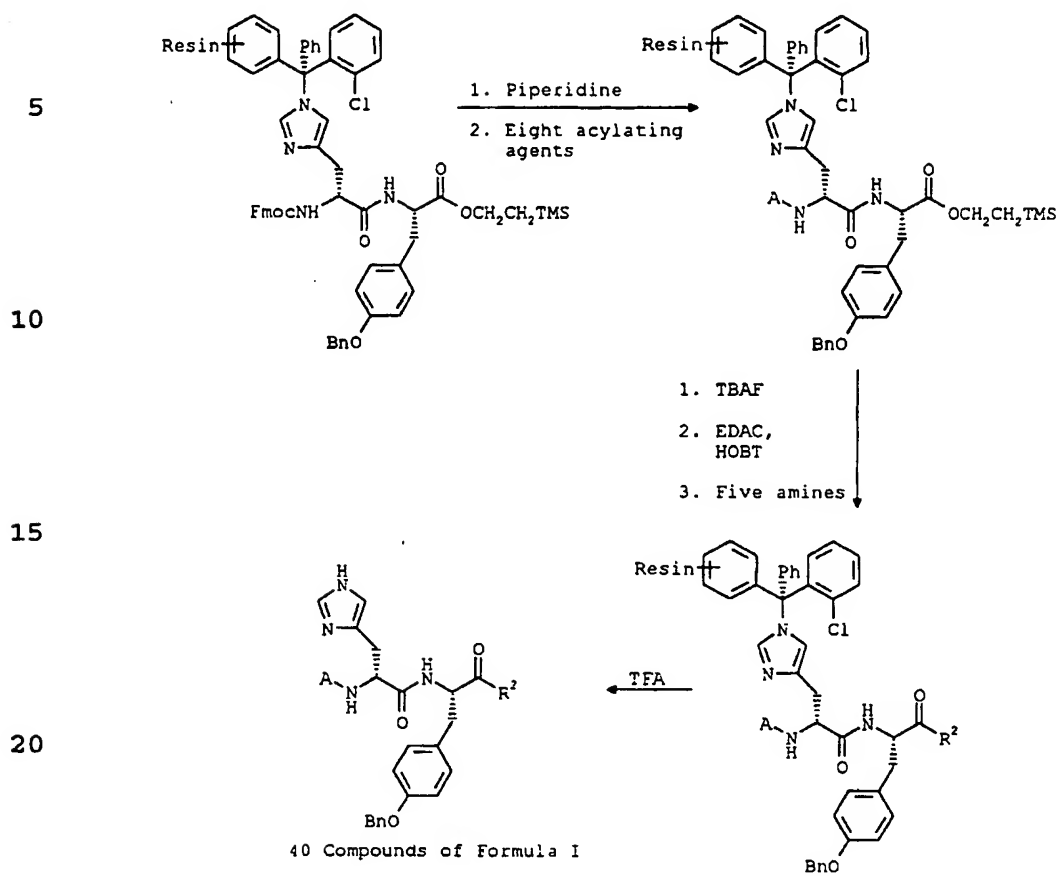
free carboxy terminus is activated with a carbodiimide reagent such as EDAC, DCC, or DIC, the activated carboxyl group is reacted with alcohols such as HOBT, NHOS, or pentachlorophenol to give an activated ester, the activated ester is reacted with a series of amines and the resulting array of compounds of Formula I is cleaved from the resin by with hot HOAc or by treatment with TFA at room temperature.

For all three synthetic methods described above appropriate consideration is given to protection and deprotection of reactive functional groups and to the sequence of synthetic steps. Knowledge of the use of common protecting groups and strategy for the assembly of complex organic molecules are within the usual realm of expertise of a practitioner of the art of organic chemistry (see, for example: T.W. Greene and P.G.M. Wuts, Protective Groups in Organic Chemistry, John Wiley and Sons (1991); E.J. Corey and X.-M. Cheng, The Logic of Chemical Synthesis, John Wiley and Sons (1989)).

The homogeneity and composition of the resulting compounds is verified by reverse phase-high pressure liquid chromatography (RP-HPLC), capillary electrophoresis, thin layer chromatography (TLC), proton nuclear magnetic resonance spectrometry (NMR), amino acid analysis, fast atom bombardment mass spectrometry (FAB-MS) and electrospray mass spectrometry (ES-MS).

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## SCHEME I: Multiple Simultaneous Synthesis Method



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The compounds of Formula I are capable of further forming both pharmaceutically acceptable acid addition and/or base salts. All of these forms are within the scope of the present invention.

5 Pharmaceutically acceptable acid addition salts of the compounds of Formula I include salts derived from nontoxic inorganic acids such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, hydrofluoric, phosphorous, and the like, as well as the  
10 salts derived from nontoxic organic acids, such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanolic acids, hydroxy alkanolic acids, alkanedioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, etc. Such salts thus include  
15 sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, nitrate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide, acetate, trifluoroacetate, propionate, caprylate, isobutyrate, oxalate, malonate,  
20 succinate, suberate, sebacate, fumarate, maleate, mandelate, benzoate, chlorobenzoate, methylbenzoate, dinitrobenzoate, phthalate, benzenesulfonate, toluenesulfonate, phenylacetate, citrate, lactate, maleate, tartrate, methanesulfonate, and the like.  
25 Also contemplated are salts of amino acids such as arginate and the like and gluconate, galacturonate, n-methyl glucamine (see, for example, Berge S.M., et al., "Pharmaceutical Salts," Journal of Pharmaceutical Science, 66:1-19 (1977)).

30 The acid addition salts of said basic compounds are prepared by contacting the free base form with a sufficient amount of the desired acid to produce the salt in the conventional manner. Preferably a compound of Formula I can be converted to an acidic salt by  
35 treating with an aqueous solution of the desired acid, such that the resulting pH is less than 4. The

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solution can be passed through a C18 cartridge to absorb the compound, washed with copious amounts of water, the compound eluted with a polar organic solvent such as, for example, methanol, acetonitrile, and the like, and isolated by concentrating under reduced pressure followed by lyophilization. The free base form may be regenerated by contacting the salt form with a base and isolating the free base in the conventional manner or as above. The free base forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free base for purposes of the present invention.

Pharmaceutically acceptable base addition salts are formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Examples of metals used as cations are sodium, potassium, magnesium, calcium, and the like. Examples of suitable amines are N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, dicyclohexylamine, ethylenediamine, N-methylglucamine, and procaine (see, for example, Berge S.M., et al., "Pharmaceutical Salts", Journal of Pharmaceutical Science, 66:1-19 (1977)).

The base addition salts of said acidic compounds are prepared by contacting the free acid form with a sufficient amount of the desired base to produce the salt in the conventional manner. Preferably, a compound of Formula I can be converted to a base salt by treating with an aqueous solution of the desired base, such that the resulting pH is greater than 9. The solution can be passed through a C18 cartridge to absorb the compound, washed with copious amounts of water, the compound eluted with a polar organic solvent such as, for example, methanol, acetonitrile and the



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like, and isolated by concentrating under reduced pressure followed by lyophilization. The free acid form may be regenerated by contacting the salt form with an acid and isolating the free acid in the  
5 conventional manner or as above. The free acid forms differ from their respective salt forms somewhat in certain physical properties such as solubility in polar solvents, but otherwise the salts are equivalent to their respective free acid for purposes of the present  
10 invention.

Certain of the compounds of the present invention can exist in unsolvated forms as well as solvated forms, including hydrated forms. In general, the solvated forms, including hydrated forms, are  
15 equivalent to unsolvated forms and are intended to be encompassed within the scope of the present invention. Certain of the compounds of the present invention possess one or more chiral centers and each center may exist in the R(D) or S(L) configuration. The present  
20 invention includes all enantiomeric and epimeric forms as well as the appropriate mixtures thereof.

The PFT inhibitory activity of compounds of Formula I was assayed in 30 mM potassium phosphate buffer, pH 7.4, containing 7 mM DTT, 1.2 mM  $\text{MgCl}_2$ ,  
25 0.1 mM leupeptin, 0.1 mM pepstatin and 0.2 mM phenylmethylsulfonyl fluoride. Assays were performed in 96 well plates (Wallec) and employed solutions composed of varying concentrations of a compound of Formula I in 100% DMSO. Upon addition of both  
30 substrates, radiolabeled farnesyl pyrophosphate ( $[1\text{-}^3\text{H}]$ , specific activity 15-30 Ci/mmol, final concentration 0.12  $\mu\text{M}$ ) and (biotinyl)-Ahe-Tyr-Lys-Cys-Val-Ile-Met peptide (final concentration 0.1  $\mu\text{M}$ ), the enzyme reaction was started by addition of 40-fold  
35 purified rat brain farnesyl protein transferase. After incubation at 37°C for 30 minutes, the reaction was

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terminated by diluting the reaction 2.5-fold with a stop buffer containing 1.5 M magnesium acetate, 0.2 M  $\text{H}_3\text{PO}_4$ , 0.5% BSA, and strepavidin beads (Amersham) at a concentration of 1.3 mg/mL. After allowing the plate to settle for 30 minutes at room temperature, radioactivity was quantitated on a microBeta counter (model 1450, Wallac). Compounds of Formula I show  $\text{IC}_{50}$  values of 0.5 nM to 80  $\mu\text{M}$  (see data table) in this assay and are thus valuable inhibitors of protein: farnesyl transferase enzyme which may be used in the medical treatment of tissue proliferative diseases, including cancer and restenosis.

15  $\text{IC}_{50}$  Values for Selected Compounds of  
Formula I Against PFT

	Example Number	$\text{IC}_{50}$ ( $\mu\text{M}$ )
	1	4.4
	4	1.0
	5 (3)	2.1
20	5 (4)	7.3
	5 (23)	0.64
	5 (27)	30
	5 (28)	0.73
	5 (30)	73
25	5 (31)	0.76
	5 (35)	66
	5 (36)	1.9
	5 (46)	1.0
	5 (49)	2.9
30	5 (40)	0.75
	5 (52)	1.6
	5 (56)	1.1
	5 (59)	20
	5 (60)	1.4

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IC<sub>50</sub> Values for Selected Compounds of  
Formula I Against PFT

	Example Number	IC <sub>50</sub> (μM)
5	5 (61)	7.2
	5 (62)	1.5
	5 (63)	1.0
	5 (64)	1.7
	5 (69)	0.48
10	5 (79)	3.0
	5 (80)	1.6
	6	0.42
	7	0.26
	8	0.074
15	9	0.27
	10	0.10
	11	0.17
	12	0.028
	13	0.083
20	15	30
	16	0.60
	17	0.039
	18	0.82
	19	0.31
25	21	0.31
	22	0.37
	23	1.9

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IC<sub>50</sub> Values for Selected Compounds of  
Formula I Against PFT (cont'd)

	Example Number	IC50 (μM)
	24	1.0
5	25	3.7
	28	11
	29	3.0

10

The compounds of the present invention can be prepared and administered in a wide variety of oral, rectal and parenteral dosage forms. Thus, the compounds of the present invention can be administered by injection, that is, intravenously, intramuscularly, intracutaneously, subcutaneously, intraduodenally, or intraperitoneally. Also, the compounds of the present invention can be administered by inhalation, for example, intranasally. Additionally, the compounds of the present invention can be administered transdermally. It will be obvious to those skilled in the art that the following dosage forms may comprise as the active component, either a compound of Formula I or a corresponding pharmaceutically acceptable salt of a compound of Formula I.

25

For preparing pharmaceutical compositions from the compounds of the present invention, pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more substances which may also act as diluents, flavoring agents, binders, preservatives, tablet disintegrating agents, or an encapsulating material.

30

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In powders, the carrier is a finely divided solid which is in a mixture with the finely divided active component.

5 In tablets, the active component is mixed with the carrier having the necessary binding properties in suitable proportions and compacted in the shape and size desired.

10 The powders and tablets preferably contain from 5 or 10 to about 70 percent of the active compound. Suitable carriers are magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, starch, gelatin, tragacanth, methylcellulose, sodium carboxymethylcellulose, a low melting wax, cocoa butter, and the like. The term "preparation" is  
15 intended to include the formulation of the active compound with encapsulating material as a carrier providing a capsule in which the active component with or without other carriers, is surrounded by a carrier, which is thus in association with it. Similarly,  
20 cachets and lozenges are included. Tablets, powders, capsules, pills, cachets, and lozenges can be used as solid dosage forms suitable for oral administration.

25 For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides or cocoa butter, is first melted and the active component is dispersed homogeneously therein, as by stirring. The molten homogenous mixture is then poured into convenient sized molds, allowed to cool, and thereby to solidify.

30 Liquid form preparations include solutions, suspensions, and emulsions, for example, water or water propylene glycol solutions. For parenteral injection liquid preparations can be formulated in solution in aqueous polyethylene glycol solution.

35 Aqueous solutions suitable for oral use can be prepared by dissolving the active component in water

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and adding suitable colorants, flavors, stabilizing and thickening agents as desired.

5       Aqueous suspensions suitable for oral use can be made by dispersing the finely divided active component in water with viscous material, such as natural or synthetic gums, resins, methylcellulose, sodium carboxymethylcellulose, and other well-known suspending agents.

10       Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavors,  
15       stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

20       The pharmaceutical preparation is preferably in unit dosage form. In such form the preparation is subdivided into unit doses containing appropriate quantities of the active component. The unit dosage form can be a packaged preparation, the package containing discrete quantities of preparation, such as packeted tablets, capsules, and powders in vials or  
25       ampoules. Also, the unit dosage form can be a capsules, tablet, cachet, or lozenge itself, or it can be the appropriate number of any of these in packaged form.

30       The quantity of active component in a unit dose preparation may be varied or adjusted from 0.1 mg to 100 mg preferably 0.5 mg to 100 mg according to the particular application and the potency of the active component. The composition can, if desired, also contain other compatible therapeutic agents.

35       In therapeutic use as inhibitors of PFT, the compounds utilized in the pharmaceutical methods of

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this invention are administered at the initial dosage of about 0.01 mg/kg to about 20 mg/kg daily. A daily dose range of about 0.01 mg/kg to about 10 mg/kg is preferred. The dosages, however, may be varied depending upon the requirements of the patient, the severity of the condition being treated, and the compound being employed. Determination of the proper dosage for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages which are less than the optimum dose of the compound. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day, if desired.

The following nonlimiting examples illustrate the inventors' preferred methods for preparing the compounds of the invention. For added clarity, complex chemical names describing compounds of Formula I are followed by structural abbreviations, which are shown in braces, wherein the structural elements are as defined in the Table of Abbreviations above.

## EXAMPLE 1

N<sub>α</sub>-[N-[N-[(Phenylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serinamide {Cbz-His-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>}

Using an ABI model 431A solid phase peptide synthesizer, Fmoc-NH<sub>2</sub>-Rink resin (0.25 mmol scale) was treated with 20% piperidine in NMP to afford NH<sub>2</sub>-Rink resin. Sequential coupling of Fmoc-protected Ser(OBn) and Tyr(OBn) (DCC and HOBT in NMP) and Fmoc deprotection (20% piperidine in NMP) reactions were run using a 4-fold excess of reagents in the coupling steps and traditional resin washing cycles to afford Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>-Rink resin. This dipeptide

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resin was transferred to an uninstrumented reaction vessel and treated with a 4-fold excess of Cbz-His, DCC, and HOBT in DMF, shaking overnight at room temperature. After removal of excess reagents, the  
5 resulting substituted tripeptide was cleaved from the resin by treatment with 50% TFA in DCM at room temperature for 2.5 hours. Evaporation of solvents and purification by reversed phase chromatography (C<sub>18</sub>-column, eluted with a 20-70% gradient of MeCN in  
10 water (both solvents acidified with 0.1% TFA) afforded Cbz-His-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub> as its TFA salt upon lyophilization. ES-MS: 719 (m+1).

Using analogous methods the following most preferred compounds of Formula I with carboxamides at  
15 the C-terminus may be prepared:

Cbz-D-His-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;  
Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;  
Cbz-D-His(1-Me)-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;  
Cbz-His-DL-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;  
20 BnNHCO-His-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>; and  
BnNHCO-D-His-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>.

## EXAMPLE 2

N-[N-[N-[(Phenylmethoxy)carbonyl]-L-histidyl]-  
25 O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serine  
{Cbz-His-Tyr(OBn)-Ser(OBn)}

Beginning with PAM resin or HMP resin, Fmoc-Ser(OBn), Fmoc-Tyr(OBn), and Cbz-His are sequentially coupled using the deprotection and  
30 coupling conditions described in Example 1. Cleavage from the resin is accomplished by treatment with CF<sub>3</sub>SO<sub>2</sub>H for the PAM supported tripeptide or with 50% TFA in DCM for the HMP supported tripeptide. Chromatography as in Example 1 provides  
35 Cbz-His-Tyr(OBn)-Ser(OBn) as its TFA salt upon



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lyophilization. See also Example 7 for a solution phase method.

Using analogous methods the following most preferred compounds of Formula I with a free carboxyl terminus may be prepared:

- 5           Cbz-D-His-Tyr(OBn)-Ser(OBn);  
          Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn);  
          Cbz-D-His(1-Me)-Tyr(OBn)-Ser(OBn);  
          Cbz-His-DL-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn);  
10          BnNHCO-His-Tyr(OBn)-Ser(OBn); and  
          BnNHCO-D-His-Tyr(OBn)-Ser(OBn).

## EXAMPLE 3

15       Solid phase supported N-[N-[(9H-Fluoren-9-ylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-L-tyrosine, 2-trimethylsilylethyl ester {Fmoc-His(2-Cl-Tr Resin)-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS}

Step 1: Boc-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS

20       2-Trimethylsilyl ethanol (2.6 g, 22.6 mmol) was added to a premixed solution of EDAC (4.3 g, 22.6 mmol), DMAP (0.5 g), and Boc-Tyr(OBn)-OH (7.0 g, 18.8 mmol) in dry THF (25 mL). The resulting mixture was stirred for 18 hours at room temperature. The solution was diluted with 1:1 EtOAc:Et<sub>2</sub>O (40 mL),  
25       washed with saturated aqueous NaHCO<sub>3</sub> (2 x 10 mL) and with saturated aqueous NaCl (2 x 10 mL), dried (MgSO<sub>4</sub>), filtered and concentrated in vacuo to provide an oil which was further purified by flash chromatography (SiO<sub>2</sub>, EtOAc:hexane eluent) to give the pure TMS-ethyl  
30       ester as an oil;

<sup>1</sup>H NMR (HCDCl<sub>3</sub>):  $\delta$  0.04 (s, 9H), 1.43 (s, 9H), 3.03 (m, 2H), 4.22 (m, 2H), 4.51 (m, 1H), 4.95 (m, 1H), 5.05 (s, 2H), 6.85-7.48 (m, 9H).

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Step 2: Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS

Eighty percent TFA in CH<sub>2</sub>Cl<sub>2</sub> (20 mL, v/v) was added to an ice-cooled solution of Boc-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS (14.8 g, 31.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (40 mL). The resulting mixture was stirred for 1.0 minute before concentrating in vacuo. The procedure was repeated once more, and the resulting residue was diluted with CH<sub>2</sub>Cl<sub>2</sub> and saturated aqueous NaHCO<sub>3</sub>. The resulting mixture was filtered through celite. The organic layer was then separated, washed with saturated aqueous NaCl, and dried (MgSO<sub>4</sub>). Filtration and concentration in vacuo provided an oil which was further purified by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent) to give the desired product;

<sup>1</sup>H NMR (HCDCl<sub>3</sub>): δ 0.06 (s, 9H), 1.68 (br s, 2H), 2.85-3.03 (m, 2H), 3.67 (m, 1H), 4.22 (m, 2H), 5.05 (s, 2H), 6.91-7.45 (m, 9H).

Step 3: Fmoc-His(Tr)-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS

To a solution of HOBT (2.6 g, 19.3 mmol) in DMF (10 mL) was added Fmoc-His(Tr) (10.0 g, 16.1 mmol) followed by EDAC (3.7 g, 19.3 mmol). The mixture was stirred at room temperature for 20 minutes before adding a solution of Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS (from Step 2 above, 5.8 g, 16.1 mmol) in DMF (10 mL). The mixture was stirred overnight at room temperature before partitioning between a mixture of water and 1:1 Et<sub>2</sub>O:EtOAc (50 mL). The layers were separated, and the organic phase was washed with saturated aqueous NaCl (4 x 20 mL) and dried (MgSO<sub>4</sub>). Filtration and concentration in vacuo provided an oil which was further purified by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent) to give the protected His-Tyr dipeptide; FAB-MS 974 (m+1).

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Step 4: Fmoc-His-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS

Fmoc-His(Tr)-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS (from Step 3 above, 5.0 g, 5.1 mmol) was treated with pyridine·OHCl (1.0 g) in MeOH (20 mL). The mixture was allowed to stir 8 hours at 65°C. The solution was concentrated in vacuo, and the residue was dissolved in CH<sub>2</sub>Cl<sub>2</sub>, washed with H<sub>2</sub>O (1x), saturated aqueous NaHCO<sub>3</sub> (2x), and dried (MgSO<sub>4</sub>). Filtration and concentration in vacuo provided an oil which was further purified by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent) to give Fmoc-His-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>STMS as a white solid; FAB-MS 731 (m+1).

Step 5: Fmoc-His(2-Cl-Tr Resin)-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS

To a suspension of Fmoc-His-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS (from Step 4 above, 5.3 g, 7.3 mmol) in CHCl<sub>3</sub> (20 mL) was added 2-chlorotrityl chloride resin (Novabiochem) (7.1 g) followed by DIEA (0.96 g, 7.4 mmol). The resulting mixture was subjected to brief sonication to disperse the resin and then agitated on a shaker for 2.0 hours. The modified resin was collected by filtration, washed with DMF (2x), MeOH (2x), CHCl<sub>2</sub> (2x), and dried in vacuo for 18 hours to yield 10.5 g (loading corresponds to approximately 1 mmol/g resin).

## EXAMPLE 4

N-[3-Phenoxypropyl]-O-(phenylmethyl)-N<sub>α</sub>-[N-  
[[ (phenylmethyl)amino]carbonyl]-L-histidyl]-L-  
tyrosinamide {BnNHCO-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh}

Fmoc-His(2-Cl-Tr-Resin)-Tyr(OBn)-CO<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>TMS (from Example 3 above, 2.0 g) was suspended in 20% piperidine in DMF. The resulting suspension was subjected to sonication for 10 minutes and then agitated by shaking for 30 minutes. The resin was filtered and washed with DMF (3x). The resin was again subjected to the same reaction conditions for an

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additional 20 minutes. The resin was filtered and washed with DMF (4x) and  $\text{CHCl}_3$  (3x) to provide His(2-Cl-Tr-Resin)-Tyr(OBn)- $\text{CO}_2\text{CH}_2\text{CH}_2\text{TMS}$  which was suspended in DCM (10 mL), agitated by shaking for 30 minutes, treated with benzyl isocyanate (1.1 g, 8.0 mmol), and agitated for an additional 30 minutes. The resin was filtered, washed with DCM (3x), resuspended in DCM, and the benzyl isocyanate treatment was repeated. The resin was filtered and washed with DMF (2x) and  $\text{CHCl}_3$  (5x) to give BnNHCO-His(2-Cl-Tr-Resin)-Tyr(OBn)- $\text{CO}_2\text{CH}_2\text{CH}_2\text{TMS}$  which was next suspended in a mixture of 4:3 dioxane:MeOH (14 mL) and treated with 1.0 M TBAF in THF (2.0 mL, 2.0 mmol). The suspension was agitated by shaking for 18 hours, filtered, washed sequentially with a 2:1 mixture of dioxane and 10% citric acid (3x 10 mL), dioxane:MeOH (3x 10 mL), dioxane (3x 10 mL), and  $\text{CHCl}_3$  (3x 10 mL) to provide BnNHCO-His(2-Cl-Tr-Resin)-Tyr(OBn). The BnNHCO-His(2-Cl-Tr-Resin)-Tyr(OBn) was suspended in DMF (10 mL) and treated with a carbodiimide coupling reagent such as DIC (0.2 g, 1.6 mmol) and HOBT (0.22 g, 1.6 mmol). The resulting mixture was stirred 30 minutes and 3-phenoxypropylamine (0.24 g, 1.6 mmol) was added. The resulting mixture was shaken 18 hours before filtering the resin and washing with DMF (3x) and  $\text{CHCl}_3$  (3x). The resin was suspended in DMF (10 mL) and the carbodiimide/HOBT/3-phenoxypropylamine coupling reaction was repeated. After 18 hours, the resin was filtered and washed with 10 mL each of MeOH (2x), DCM (3x), DMF (2x), MeOH (2x), and  $\text{CHCl}_3$  (2x) to give BnNHCO-His(2-Cl-Tr-Resin)-Tyr(OBn)- $\text{CONH}(\text{CH}_2)_3\text{OPh}$ . The highly substituted dipeptide was cleaved from the resin by treatment with 40% TFA in DCM, shaking for 1 hour at room temperature. The supernate, containing the free dipeptide, was filtered away from the resin and the resin was washed with DCM (6x). The combined supernate

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and washings were concentrated in vacuo to provide BnNHCO-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh·TFA. The product was partitioned between water and DCM, and both layers were treated dropwise with saturated aqueous NaHCO<sub>3</sub> until the aqueous layer remained basic. The layers were separated, and the organic phase was washed with saturated aqueous NaCl and dried (MgSO<sub>4</sub>). Filtration and concentration yielded BnNHCO-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh; ES-MS 675 (m+1).

## EXAMPLE 5

Multiple, Simultaneous Solid Phase Synthesis

The method described in Example 4 may be employed in simultaneous multiple syntheses using the Diversomer apparatus described by S.H. DeWitt, et al., Proc. Natl. Acad. Sci. USA, 90:6909 (1993). Fmoc-D-His(2-Cl-Tr Resin)-Tyr(OBn)-CO<sub>2</sub>iPr, prepared according to from Example 3 by substituting Fmoc-D-His(Tr) for Fmoc-His(Tr) in Step 3, (100-200 mg) is placed in each of 40 gas dispersion tubes, and the tubes are placed in the Diversomer apparatus. The sequential deprotection and coupling reactions described in Example 4 are followed, employing the following acylating agents and amines in various combinations:

Acylating agentsAmines

- |                               |                                       |
|-------------------------------|---------------------------------------|
| 1) benzyl isocyanate          | 1) 3-phenoxypropylamine               |
| 2) p-toluenesulfonyl chloride | 2) 2-(phenylmethoxy)ethyl amine       |
| 3) cyclohexyl isocyanate      | 3) 2-[(phenylmethyl)-thio]-ethylamine |
| 4) phenyl isocyanate          | 4) 4-phenylbutylamine                 |
| 5) i-propyl isocyanate        | 5) 3-(2-methoxyphenyl)-propylamine    |
| 6) n-butyl isocyanate         | 6) 1-benzyl piperazine                |
| 7) 4-chlorophenyl isocyanate  | 7) o-benzyl-hydroxylamine             |
| 8) 1-naphthyl isocyanate      | 8) methionine methyl ester            |
| 9) 3-methoxypropyl isocyanate | 9) benzylamine                        |
| 10) 4-ethoxyphenyl isocyanate | 10) 2-phenylethylamine                |
| 11) 2-phenethyl isocyanate    |                                       |

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- 12) 3-phenylpropionyl chloride
- 14) phenylacetyl chloride
- 15) 4-phenoxyphenyl isocyanate
- 16) benzyl chloroformate
- 5 17) (trans)-2-phenylcyclopropyl isocyanate
- 18) 1-adamantyl chloroformate

Array 1. Following cleavage from the resin and work-up as described in Example 4, the following substituted dipeptides (1-40) of Formula I are prepared:

1. PhNHCO-D-His-Tyr (OBn) -CONHCH<sub>2</sub>CH<sub>2</sub>OBn
2. PhNHCO-D-His-Tyr (OBn) -CONHCH<sub>2</sub>CH<sub>2</sub>SBn ES-MS 677 (m+1)
3. PhNHCO-D-His-Tyr (OBn) -CONHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OPh ES-MS 661 (m+1)
4. BuNHCO-D-His-Tyr (OBn) -CONH(CH<sub>2</sub>)<sub>4</sub>Ph ES-MS 639 (m+1)
- 15 5. BuNHCO-D-His-Tyr (OBn) -CO(4-Bn-piperazin-1-yl) ES-MS 665 (m)
6. (4-MePh)SO<sub>2</sub>-D-His-Tyr (OBn) -CONHCH<sub>2</sub>CH<sub>2</sub>Ph ES-MS 666 (m+1)
7. (4-MePh)SO<sub>2</sub>-D-His-Tyr (OBn) -CONHCH<sub>2</sub>CH<sub>2</sub>SBn ES-MS 712 (m+1)
8. CF<sub>3</sub>SO<sub>2</sub>-D-His-Tyr (OBn) -CONH-Met-CO<sub>2</sub>Me
- 20 9. CF<sub>3</sub>SO<sub>2</sub>-D-His-Tyr (OBn) -CONHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>-(2-MeO-Ph)
10. CF<sub>3</sub>SO<sub>2</sub>-D-His-Tyr (OBn) -CO(4-Bn-piperazin-1-yl)
11. BnNHCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>CH<sub>2</sub>Ph
12. MeO(CH<sub>2</sub>)<sub>3</sub>NHCO-D-His-Tyr (OBn) CONHOBn
13. MeO(CH<sub>2</sub>)<sub>3</sub>NHCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OPh
- 25 14. MeO(CH<sub>2</sub>)<sub>3</sub>NHCO-D-His-Tyr (OBn) CONH(CH<sub>2</sub>)<sub>3</sub>-(2-MeO-Ph)
15. BnNHCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>Ph ES-MS 631 (m+1)
16. (4-ClPh)NHCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>CH<sub>2</sub>OBn ES-MS 695 (m+1)
17. 1-Napthyl-NHCO-D-His-Tyr (OBn) CONHOBn ES-MS 683 (m+1)
- 30 18. 1-Napthyl-NHCO-D-His-Tyr (OBn) CONH-Met-CO<sub>2</sub>Me ES-MS 723 (m+1)
19. (4-ClPh)NHCO-D-His-Tyr (OBn) CONH(CH<sub>2</sub>)<sub>4</sub>Ph ES-MS 693 (m+1)
20. (4-ClPh)NHCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>Ph ES-MS 651 (m+1)
21. BnOCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>CH<sub>2</sub>OBn ES-MS 676 (m+1)
22. 1-adamantyl-OCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>CH<sub>2</sub>SBn
- 35 23. BnOCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OPh ES-MS 676 (m+1)
24. 1-adamantyl-OCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>Ph
25. BnOCO-D-His-Tyr (OBn) CO(4-Bn-piperazin-1-yl) ES-MS 700 (m)
- 40 26. PhCH<sub>2</sub>CO-D-His-Tyr (OBn) CONHCH<sub>2</sub>CH<sub>2</sub>Ph ES-MS 630 (m+1)
27. PhCH<sub>2</sub>CH<sub>2</sub>NHCO-D-His-Tyr (OBn) CONHCH<sub>2</sub>CH<sub>2</sub>SBn ES-MS 705 (m+1)

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	28.	PhCH <sub>2</sub> CH <sub>2</sub> NHCO-D-His-Tyr (OBn) CONH-Met-CO <sub>2</sub> Me	ES-MS 701 (m+1)
	29.	PhCH <sub>2</sub> CH <sub>2</sub> NHCO-D-His-Tyr (OBn) CONH(CH <sub>2</sub> ) <sub>3</sub> -(2-MeO-Ph)	ES-MS 703 (m+1)
5	30.	PhCH <sub>2</sub> CO-D-His-Tyr (OBn) CO(4-Bn-piperazin-1-yl)	ES-MS 684 (m)
	31.	(t-2-Ph-c-propyl)-NHCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> Ph	ES-MS 671 (m+1)
10	32.	(t-2-Ph-c-propyl)-NHCO-D-His-Tyr (OBn) CONHOBn	ES-MS 673 (m+1)
	33.	c-hexyl-NHCO-D-His-Tyr (OBn) CONH(CH <sub>2</sub> ) <sub>3</sub> OPh	ES-MS 666 (m+1)
	34.	c-hexyl-NHCO-D-His-Tyr (OBn) CONH(CH <sub>2</sub> ) <sub>3</sub> -(2-MeO-Ph)	ES-MS 681 (m+1)
15	35.	c-hexyl-NHCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> Ph	ES-MS 623 (m+1)
	36.	PhCH <sub>2</sub> CH <sub>2</sub> CO-D-His-Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> OBn	ES-MS 674 (m+1)
	37.	PhCH <sub>2</sub> CH <sub>2</sub> CO-D-His-Tyr (OBn) CONHOBn	ES-MS 646 (m+1)
	38.	(CH <sub>3</sub> ) <sub>2</sub> CHNHCO-D-His-Tyr (OBn) CONH-Met-CO <sub>2</sub> Me	ES-MS 639 (m+1)
20	39.	PhCH <sub>2</sub> CH <sub>2</sub> CO-D-His-Tyr (OBn) CONH(CH <sub>2</sub> ) <sub>3</sub> Ph	ES-MS 672 (m+1)
	40.	(CH <sub>3</sub> ) <sub>2</sub> CHNHCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> Ph	ES-MS 583 (m+1)

Array 2. Following cleavage from the resin and work-up as described in Example 4, the following substituted dipeptides (41-80) of Formula I are prepared:

25	41.	n-BuNHCO-D-His-Tyr (OBn) -CONHCH <sub>2</sub> CH <sub>2</sub> OBn	ES-MS 641 (m+1)
	42.	n-BuNHCO-D-His-Tyr (OBn) -CONHCH <sub>2</sub> CH <sub>2</sub> SBn	ES-MS 657 (m+1)
	43.	n-BuNHCO-D-His-Tyr (OBn) -CONHCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> OPh	ES-MS 641 (m+1)
	44.	PhNHCO-D-His-Tyr (OBn) -CONHCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> Ph	ES-MS 659 (m+1)
30	45.	PhNHCO-D-His-Tyr (OBn) -CO(4-Bn-piperazin-1-yl)	ES-MS 685 (m)
	46.	(4-PhOPh)NHCO-D-His-Tyr (OBn) -CONHCH <sub>2</sub> CH <sub>2</sub> Ph	ES-MS 723 (m+1)
	47.	(4-PhOPh)NHCO-D-His-Tyr (OBn) -CONHCH <sub>2</sub> CH <sub>2</sub> SBn	ES-MS 769 (m+1)
	48.	(4-MePh)SO <sub>2</sub> -D-His-Tyr (OBn) -CONH-Met-CO <sub>2</sub> Me	ES-MS 708 (m+1)
	49.	(4-MePh)SO <sub>2</sub> -D-His-Tyr (OBn) -CONHCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> -(2-MeO-Ph)	
35	50.	(4-MePh)SO <sub>2</sub> -D-His-Tyr (OBn) -CO(4-Bn-piperazin-1-yl)	ES-MS 720 (m)
	51.	MeO(CH <sub>2</sub> ) <sub>3</sub> NHCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> Ph	
	52.	BnNHCO-D-His-Tyr (OBn) CONHOBn	ES-MS 647 (m+1)
40	53.	BnNHCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> OPh	ES-MS 675 (m+1)

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	54.	BnNHCO-D-His-Tyr (OBn) CONH(CH <sub>2</sub> ) <sub>3</sub> - (2-MeO-Ph)	ES-MS 689 (m+1)
	55.	MeO(CH <sub>2</sub> ) <sub>3</sub> NHCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> Ph	
	56.	1-naphthyl-NHCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> OBn	ES-MS 711 (m+1)
5	57.	(4-ClPh) NHCO-D-His-Tyr (OBn) CONHOBn	
	58.	(4-ClPh) NHCO-D-His-Tyr (OBn) CONH- Met-CO <sub>2</sub> Me	ES-MS 707 (m+1)
	59.	1-naphthyl-NHCO-D-His- Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> Ph	ES-MS 709 (m+1)
10	60.	1-naphthyl-NHCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> Ph	
	61.	(4-EtOPh) NHCO-D-His- Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> OBn	ES-MS 705 (m+1)
	62.	BnOCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> SBn	
15	63.	(4-EtOPh) NHCO-His- Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> OPh	ES-MS 705 (m+1)
	64.	BnOCO-D-His-Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> Ph	
	65.	(4-EtOPh) NHCO-D-His-Tyr (OBn) CO(4-Bn- piperazin-1-yl)	ES-MS 729 (m)
	66.	PhCH <sub>2</sub> CO-D-His-Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> Ph	ES-MS 659 (m+1)
20	67.	PhCH <sub>2</sub> CO-D-His-Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> SBn	ES-MS 676 (m+1)
	68.	PhCH <sub>2</sub> CO-D-His-Tyr (OBn) CONH-Met-CO <sub>2</sub> Me	
	69.	PhCH <sub>2</sub> CO-D-His-Tyr (OBn) CONH(CH <sub>2</sub> ) <sub>3</sub> - (2-MeO-Ph)	ES-MS 674 (m+1)
	70.	PhCH <sub>2</sub> CH <sub>2</sub> NHCO-D-His-Tyr (OBn) CO(4-Bn- piperazin-1-yl)	ES-MS 713 (m)
25	71.	c-hexyl-NHCO-D-His- Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> Ph	ES-MS 637 (m+1)
	72.	c-hexyl-NHCO-D-His-Tyr (OBn) CONHOBn	ES-MS 639 (m+1)
	73.	(t-2-Ph-c-propyl)-NHCO-D-His- Tyr (OBn) CONH(CH <sub>2</sub> ) <sub>3</sub> OPh	ES-MS 701 (m+1)
30	74.	(t-2-Ph-c-propyl)-NHCO-D-His- Tyr (OBn) CONH(CH <sub>2</sub> ) <sub>3</sub> -(2-MeO-Ph)	
	75.	(t-2-Ph-c-propyl)-NHCO-D-His- Tyr (OBn) CONHCH <sub>2</sub> Ph	ES-MS 657 (m+1)
35	76.	(CH <sub>3</sub> ) <sub>2</sub> CHNHCO-D-His- Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> OBn	ES-MS 627 (m+1)
	77.	(CH <sub>3</sub> ) <sub>2</sub> CHNHCO-D-His-Tyr (OBn) CONHOBn	ES-MS 599 (m+1)
	78.	PhCH <sub>2</sub> CH <sub>2</sub> CO-D-His-Tyr (OBn) CONH-Met-CO <sub>2</sub> Me	ES-MS 686 (m+1)
	79.	(CH <sub>3</sub> ) <sub>2</sub> CHNHCO-D-His- Tyr (OBn) CONHCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> Ph	ES-MS 625 (m+1)
40	80.	PhCH <sub>2</sub> CH <sub>2</sub> CO-D-His-Tyr (OBn) CONHCH <sub>2</sub> Ph	ES-MS 630 (m+1)



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## EXAMPLE 6

N-[N-[N-[(Phenylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serine methyl ester {Cbz-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me}

5 Step 1: Boc-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

To a solution of Boc-Tyr(OBn) (1.88 g, 6.50 mmol) in EtOAc (30 mL) at 0°C was added HOBT hydrate (1.19 g, 7.80 mmol) followed by DCC (1.61 g, 7.80 mmol). A solution of Ser(OBn)-CO<sub>2</sub>Me·TFA (2.1 g, 6.50 mmol) in EtOAc (20 mL) was added followed by Et<sub>3</sub>N (1.09 mL, 7.80 mmol). The mixture was allowed to warm to room temperature and stirred overnight. The mixture was filtered, diluted with EtOAc, and washed twice with saturated aqueous NaHCO<sub>3</sub>, brine, dried over MgSO<sub>4</sub>, and concentrated. Flash chromatography (40% EtOAc/hexane) gave 2.67 g (73%) of the title compound as a white solid, mp 81-84°C.

Step 2: Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me·TFA

20 Boc-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me (from Step 1 above, 2.64 g, 4.69 mmol) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (15 mL), cooled to 0°C and TFA (5 mL) was added. The solution was warmed to room temperature and stirred for 4 hours. The solution was concentrated, taken up in CH<sub>2</sub>Cl<sub>2</sub> and reconcentrated twice. The resulting oil was triturated with ether to provide 2.7 g of the title compound as a white solid.

Step 3: Cbz-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

30 To a solution of Cbz-His (1.00 g, 3.47 mmol) in DMF (15 mL) at 0°C was added HOBT (0.64 g, 4.16 mmol) and DCC (0.86 g, 4.16 mmol). Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me·TFA (from Step 2 above, 2.0 g, 3.47 mmol) was added followed by Et<sub>3</sub>N (0.58 mL, 4.16 mmol). The mixture was allowed to warm to room temperature and stirred overnight. The mixture was filtered and the

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filtrate was diluted with  $\text{CHCl}_3$ , washed twice with saturated aqueous  $\text{NaHCO}_3$ , brine, dried over  $\text{MgSO}_4$ , and concentrated. Flash chromatography (2-5%  $\text{MeOH}/\text{CHCl}_3$ ) gave 2.14 g of the title compound as a white solid,

mp 175-176°C; FAB-MS 734 ( $m+1$ );

Anal. Calc. for  $\text{C}_{41}\text{H}_{43}\text{N}_5\text{O}_8$ :

C, 67.11; H, 5.91; N, 9.54;

Found: C, 66.96; H, 6.01; N, 9.41.

10

## EXAMPLE 7

N-[N-[N-[(Phenylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serine {Cbz-His-Tyr(OBn)-Ser(OBn)}

To a solution of Cbz-His-Tyr(OBn)-Ser(OBn)- $\text{CO}_2\text{Me}$  (from Example 6 above, 2.02 g, 2.75 mmol) in THF (50 mL) and MeOH (15 mL) at 0°C was added 0.1N LiOH (30.3 mL, 3.03 mmol). The solution was stirred for 6 hours at 0°C, then concentrated. Water was added and the pH was adjusted to 4-5 with 1N HCl. The mixture was filtered, and the solid was collected and dried to provide 1.55 g (78%) of the title compound as a white solid, mp 187-192°C; ES-MS 720 ( $m+1$ );

Anal. Calc. for  $\text{C}_{40}\text{H}_{41}\text{N}_5\text{O}_8 \cdot 1.5\text{H}_2\text{O}$ :

C, 64.33; H, 5.94; N, 9.38;

Found: C, 64.29; H, 5.73; N, 9.15.

25

## EXAMPLE 8

N-[N-[N-[(Phenylmethoxy)carbonyl]-D-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serine, methyl ester {Cbz-D-His-Tyr(OBn)-Ser(OBn)- $\text{CO}_2\text{Me}$ }

30

Step 1: Tyr(OBn)-Ser(OBn)- $\text{CO}_2\text{Me} \cdot \text{HCl}$

A solution of Boc-Tyr(OBn)-Ser(OBn)- $\text{CO}_2\text{Me}$  (from Example 6, Step 1 above, 9.90 g, 17.6 mmol) in EtOAc was cooled to 0°C. Anhydrous HCl gas was bubbled through the cold solution for 5 minutes. The solution was allowed to warm to room temperature and stirred

35

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overnight. The solution was concentrated, taken up in EtOAc and reconcentrated to provide 8.75 g of the title compound as a foam; CI-MS 463 (m+1).

5     Step 2: Cbz-D-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

According to Example 6, Step 3, by substituting Cbz-D-His(Tr) for Cbz-His and Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me·HCl for Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me·TFA, the title compound was obtained as a white solid, mp 78-88°C; FAB-MS 976 (m+1).

Step 3: Cbz-D-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

A solution of Cbz-D-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me (from Step 2 above, 0.27 g, 0.28 mmol) in HOAc:H<sub>2</sub>O (4:1, 2 mL) was stirred at 80°C for 5 minutes, then cooled to room temperature. The solution was partitioned between EtOAc and saturated aqueous NaHCO<sub>3</sub>. The organic layer was washed with brine, dried (MgSO<sub>4</sub>), and concentrated. Flash chromatography (2-5% MeOH:CHCl<sub>3</sub>) yielded 0.10 g of the title compound as a foam; FAB-MS 734 (m+1).

EXAMPLE 9

25     N-[N-[N-[(Phenylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-D-serine, methyl ester {Cbz-His-Tyr(OBn)-D-Ser(OBn)-CO<sub>2</sub>Me}

According to Example 6, by substituting D-Ser(OBn)-CO<sub>2</sub>Me·TFA for Ser(OBn)-CO<sub>2</sub>Me·TFA in Step 1, the title compound was obtained, mp 168-170°C; FAB-MS 734 (m+1).

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## EXAMPLE 10

N-[ $\alpha$ -Methyl-N-[N-[(phenylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-DL-tyrosyl]-O-(phenylmethyl)-L-serine, methyl ester {Cbz-His-DL-( $\alpha$ -Me)Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me}

5 According to Example 6, by substituting Boc-DL-( $\alpha$ -Me)Tyr(OBn) for Boc-Tyr(OBn) in Step 1, the title compound was obtained; FAB-MS 748 (m+1).

## EXAMPLE 11

10 N-Ethyl-N<sub>α</sub>-[N-[N-[(phenylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serinamide {Cbz-His-Tyr(OBn)-Ser(OBn)-CONHET}

According to Example 6, by substituting Boc-Tyr(OBn)-Ser(OBn)-CONHET for Boc-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me in Step 2, the title compound was  
15 obtained, mp 182-188°C; FAB-MS 747 (m+1).

## EXAMPLE 12

20 N-Ethyl-N<sub>α</sub>-[N-[N-[(Phenylmethoxy)carbonyl]-D-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serinamide {Cbz-D-His-Tyr(OBn)-Ser(OBn)-CONHET}

According to Example 6, by substituting Boc-Tyr(OBn)-Ser(OBn)-CONHET for Boc-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me in Step 2 and Cbz-D-His for Cbz-His in Step 3,  
25 the title compound was obtained, mp 193-196°C; ES-MS 747 (m+1).

## EXAMPLE 13

30 N-[N-[1-Methyl-N-[(phenylmethoxy)carbonyl]]-L-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serine, methyl ester {Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me}

Step 1: Cbz-His(1-Me)

35 Benzyl chloroformate (0.24 mL, 1.7 mmol) was added dropwise to a slurry of 1-methyl-L-histidine (0.25 g,

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1.5 mmol) in THF (5 mL) and saturated aqueous NaHCO<sub>3</sub> (5 mL) at 0°C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was concentrated and diluted with H<sub>2</sub>O, washed with ether, and the pH adjusted to 6-7 with 1N HCl. The mixture was concentrated, then diluted with CHCl<sub>3</sub> (150 mL) and MeOH (15 mL), and stirred for 1 hour. The mixture was dried (MgSO<sub>4</sub>) and concentrated to provide 0.48 g of the title compound which was used without further purification.

Step 2: Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

To a slurry of Cbz-His(1-Me) (from Step 1 above, 0.36 g, 1.2 mmol), Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me·HCl (from Example 8, Step 1 above, 0.60 g, 1.2 mmol), DCC (0.30 g, 1.4 mmol), and HOBT (0.19 g, 1.4 mmol) in CH<sub>2</sub>Cl<sub>2</sub> was added Et<sub>3</sub>N (0.17 mL, 1.2 mmol) and the mixture was stirred overnight at room temperature. The mixture was diluted with CHCl<sub>3</sub>, washed with saturated aqueous NaHCO<sub>3</sub>, brine, dried (MgSO<sub>4</sub>), and concentrated. Flash chromatography (1% MeOH:CHCl<sub>3</sub>) provided the title compound as a white solid, mp 161.5-163.5°C; FAB-MS 748 (m+1).

25

EXAMPLE 14

N-[N-[1-Methyl-N-[(phenylmethoxy)carbonyl]-D-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serine, methyl ester {Cbz-D-His(1-Me)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me}

According to Example 13, by substituting 1-methyl-D-histidine for 1-methyl-L-histidine, the title compound was obtained; FAB-MS 748 (m+1).

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## EXAMPLE 15

5 N-[L-2-Amino-N-[N-[(phenylmethoxy)carbonyl]-D-histidyl]-4-[4-(phenylmethoxy)phenyl]butanoyl]-O-(phenylmethyl-L-serine, methyl ester {Cbz-D-His-homoTyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me}

According to Example 6, by substituting Boc-homoTyr(OBn) for Boc-Tyr(OBn) in Step 1, and substituting Cbz-D-His for Cbz-His in Step 3, the title compound was obtained; ES-MS 748 (m+1).

10

## EXAMPLE 16

15 N-[4-Phenyl-N-[N-[(phenylmethoxy)carbonyl]-L-histidyl]-L-phenylalanyl]-O-(phenylmethyl-L-serine, methyl ester {Cbz-His-Phe(4-Ph)-Ser(OBn)-CO<sub>2</sub>Me}

According to Example 6, by substituting Boc-Phe(4-Ph) for Boc-Tyr(OBn) in Step 1, the title compound was obtained, mp 184-187°C; FAB-MS 704 (m+1).

## EXAMPLE 17

20 N-[O-(phenylmethyl)-N[N-[[N-[(phenylmethyl)amino]-carbonyl]-L-histidyl]-L-tyrosyl]-O-(phenylmethyl)-serine, methyl ester {BnNHCO-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me}

Step 1: Fmoc-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

25 According to Example 13, Step 2, by substituting Fmoc-His(Tr) for Cbz-His(1-Me), the title compound was obtained, mp 82-92°C.

Step 2: His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

30 Piperidine (4.0 mL) was added to a slurry of Fmoc-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me (from Step 1 above, 1.85 g, 1.74 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (20 mL). The solution was stirred for 2 hours at room temperature, then concentrated. The residue was taken up in EtOAc (150 mL), washed with water (3 x 50 mL), dried (MgSO<sub>4</sub>),  
35 and concentrated. The resulting oil was triturated

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with Et<sub>2</sub>O/hexane. Flash chromatography of the residue (2% MeOH/CHCl<sub>3</sub>) gave 1.03 g of the title compound as a foam, mp 61.5-70°C; ES-MS 843 (m+1).

5      Step 3: BnNHCO-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

        Benzyl isocyanate (0.053 mL, 0.43 mmol) was added in one portion to a solution of His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me (from Step 2 above, 0.33 g, 0.39 mmol) in EtOAc (5 mL). The resulting slurry was stirred for  
10      3 hours at room temperature, then concentrated to yield the title compound (0.4 g), which was used without further purification.

        Step 4: BnNHCO-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

15      According to Example 8, by substituting BnNHCO-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me for Cbz-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me, the title compound was obtained, mp 196.5-199°C; ES-MS 733 (m+1).

20

## EXAMPLE 18

N-[N-[N-(1-Oxo-3-phenylpropyl)-L-histidyl]-O-(phenylmethyl)-L-tyrosyl]-O-(phenylmethyl)-L-serine, methyl ester {PhCH<sub>2</sub>CH<sub>2</sub>CO-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me}

25      Step 1: PhCH<sub>2</sub>CH<sub>2</sub>CO-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

        To a cooled (0°C) solution of His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me (from Example 17, Step 2 above, 0.33 g, 0.39 mmol) in THF (5 mL) was added Et<sub>3</sub>N (0.06 mL, 0.43 mmol) followed by phenylpropionyl chloride (0.064 mL, 0.43 mmol). The resulting slurry was  
30      brought to room temperature and stirred overnight. The mixture was partitioned between EtOAc and saturated aqueous NaHCO<sub>3</sub>. The organic layer was washed with brine, dried (MgSO<sub>4</sub>), and concentrated to yield the title compound as a solid which was used without  
35      further purification.

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Step 2: PhCH<sub>2</sub>CH<sub>2</sub>CO-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me

According to Example 8, Step 3, by substituting PhCH<sub>2</sub>CH<sub>2</sub>CO-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me for Cbz-D-His(Tr)-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me, the title compound was  
5 obtained, mp 193-196.5°C; ES-MS 732 (m+1).

## EXAMPLE 19

N<sub>α</sub>-[N-[(Phenylmethoxy)carbonyl]-L-histidyl]-  
O-(phenylmethyl)-N-[2-(phenylmethoxy)ethyl]-  
10 L-tyrosinamide {Cbz-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>OBn}  
Step 1: Cbz-His-Tyr(OBn)-CO<sub>2</sub>Me

According to Example 6, Step 3, by substituting Tyr(OBn)-CO<sub>2</sub>Me·TFA for Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me·TFA, the title compound was obtained as a white powder,  
15 mp 145-148°C; CI-MS 557 (m+1).

Step 2: Cbz-His-Tyr(OBn)

According to Example 7, by substituting Cbz-His-Tyr(OBn)-CO<sub>2</sub>Me for Cbz-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me, the title compound was obtained as a white powder,  
20 mp 79-92°C; CI-MS 543 (m+1).

Step 3: Cbz-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>OBn

To a solution of Cbz-His-Tyr(OBn) (from Step 2  
25 above, 0.43 g, 0.79 mmol) in DMF (4 mL) at 0°C was added HOBT (0.15 g, 0.95 mmol) and DCC (0.20 g, 0.95 mmol). A solution of 2-(phenylmethoxy)ethylamine (0.12 g, 0.79 mmol) in DMF (1 mL) was then added. The mixture was allowed to warm to room temperature and  
30 stirred overnight. The mixture was filtered, diluted with CHCl<sub>3</sub>, washed twice with saturated aqueous NaHCO<sub>3</sub>, washed with brine, dried over MgSO<sub>4</sub>, and concentrated. Flash chromatography (3-5% MeOH/CHCl<sub>3</sub>) afforded 0.34 g (63%) of the title compound as a white solid,  
35 mp 136-150°C; FAB-MS 676 (m+1);



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Anal. Calc. for  $C_{39}H_{41}N_5O_6$ :

C, 69.32; H, 6.12; N, 10.36;

Found: C, 69.43; H, 6.24; N, 10.45.

5

## EXAMPLE 20

$N_\alpha$ -[N-[(Phenylmethoxy)carbonyl]-D-histidyl]-N-[2-(phenylmethoxy)ethyl]-O-(phenylmethyl)-L-tyrosinamide {Cbz-D-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>OBn}

10 According to Example 6, by substituting 2-(phenylmethoxy)ethylamine for Ser(OBn)·TFA and omitting Et<sub>3</sub>N in Step 1 and by substituting Cbz-D-His for Cbz-His in Step 3, the title compound was prepared, mp 161-165°C; FAB-MS 676 (m+1).

15

## EXAMPLE 21

$N_\alpha$ -[N-Methyl-N[(phenylmethoxy)carbonyl]-D-histidyl]-N-[2-(phenylmethoxy)ethyl]-O-(phenylmethyl)-L-tyrosinamide {Cbz-D-His-(N-Me)Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>OBn}

20 According to Example 20, by substituting Boc-(N-Me)Tyr(OBn) for Boc-Tyr(OBn), the title compound was obtained, mp 64-78°C; ES-MS 690 (m+1).

## EXAMPLE 22

25  $N_\alpha$ -[ $\alpha$ -Methyl-N-[N-[(phenylmethoxy)carbonyl]-D-histidyl]-N-[2-(phenylmethoxy)ethyl]-O-(phenylmethyl)-L-tyrosinamide {Cbz-D-His-( $\alpha$ -Me)Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>OBn}

30 According to Example 20, by substituting Boc-( $\alpha$ -Me)Tyr(OBn) for Boc-Tyr(OBn), the title compound was obtained, mp 66-78°C; ES-MS 690 (m+1).

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## EXAMPLE 23

N-(2-Phenylethyl)-N<sub>α</sub>-[N-[(phenylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-L-tyrosinamide  
{Cbz-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>Ph}

- 5        According to Example 19, Step 3, by substituting 2-phenylethylamine for 2-(phenylmethoxy)ethylamine, the title compound was obtained as a white solid, mp 188-189.5°C; FAB-MS 646 (m+1).

## EXAMPLE 24

10        N-[N-[N-[(Phenylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-L-tyrosyl]-3-(3-pyridinyl)-L-alanine,  
methyl ester {Cbz-His-Tyr(OBn)-Pyr-CO<sub>2</sub>Me}

- 15        According to Example 19, Step 3, by substituting Pyr-CO<sub>2</sub>Me for 2-(phenylmethoxy)ethylamine, the title compound was obtained as a white solid, mp 180-182.5°C (dec); FAB-MS 705 (m+1).

## EXAMPLE 25

20        (S,R)-N-[2-(4-Benzoyloxy-phenyl)-1-(3-phenoxy-propylcarbamoyl)-ethyl]-2-[3-(4-ethoxy-phenyl)-ureido]-3-(3H-imidazol-4-yl)-propionamide  
{(4-EtOPh)NHCO-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh}

Step 1. Boc-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh

- 25        2-(Phenylmethoxy)ethylamine (0.81 g, 5.4 mmol) was added to a premixed solution of EDAC (1.2 g, 6.5 mmol), HOBT (0.87 g, 6.5 mmol), and Boc-Tyr(OBn)-OH (2.0 g, 5.4 mmol) in dry DMF (15 mL). The resulting mixture was stirred for 18 hours at room temperature. The solution was diluted with 1:1 EtOAc:Et<sub>2</sub>O (40 mL), washed with saturated aqueous NaCl (4 x 10 mL), dried (MgSO<sub>4</sub>), filtered, and concentrated in vacuo to provide a solid which was further purified by trituration with hexane to give the pure product, mp 145-146°C.

35

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Step 2. Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh

Dry HCl gas was bubbled into an ice cold solution of Boc-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh (from Step 1 above, 2.0 g, 3.9 mmol) in MeOH (15 mL) for 4 minutes. The  
5 resulting mixture was stirred for 1 hour at 0°C and then allowed to warm to room temperature and stir 1 hour. The solution was concentrated in vacuo to provide a solid which was triturated with ether to provide Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh·HCl; CI-MS 405 (m+1).  
10 The title compound was suspended in CHCl<sub>3</sub>, cooled in an ice bath, and NH<sub>3</sub> gas was bubbled through the mixture for 2 minutes. The NH<sub>4</sub>Cl was filtered off, and the supernate was concentrated in vacuo to yield the free base of the title compound which was used in the next  
15 step without further purification.

Step 3. Fmoc-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh

To a solution of HOBT (0.48 g, 3.5 mmol) in DMF (10 mL) was added Fmoc-D-His(Tr)-CO<sub>2</sub>H (2.0 g, 3.2 mmol)  
20 followed by EDAC (0.67 g, 3.5 mmol). The mixture was stirred at room temperature for 20 minutes before adding a solution of Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh (from Step 2 above, 1.4 g, 3.2 mmol) in DMF (10 mL). The mixture was stirred overnight at room temperature  
25 before partitioning between a mixture of water (20 mL) and 1:1 Et<sub>2</sub>O:EtOAc (50 mL). The layers were separated, and the organic phase was washed with saturated aqueous NaCl (4x 20 mL) and dried (MgSO<sub>4</sub>). Filtration and concentration in vacuo provided an oil which was  
30 further purified by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>: MeOH eluent) to give the protected His-Tyr dipeptide; FAB-MS 1006 (m).

Step 4. D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh

35 Fmoc-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh (from Step 3 above, 1.0 g, 0.99 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was treated

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with piperidine (0.18 g, 2.1 mmol). The resulting mixture was stirred 2 hours before concentrating in vacuo and purifying the resulting oil by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent) to give  
 5 (4-EtOPh)NHCO-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh;  
 ES-MS 784 (m+1).

Step 5. (4-EtOPh)NHCO-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh  
 D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh (from Step 4  
 10 above, 0.55 g, 0.7 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was treated  
 with 4-ethoxyphenyl isocyanate (0.1 g, 0.7 mmol). The  
 resulting mixture was stirred 1 hour at room  
 temperature. Concentrated in vacuo. The resulting oil  
 was purified by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH  
 15 eluent) to give (4-EtOPh)NHCO-D-His(Tr)-Tyr(OBn)-  
 CONH(CH<sub>2</sub>)<sub>3</sub>OPh; ES-MS 947 (m+1).

Step 6. (4-EtOPh)NHCO-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh  
(4-EtOPh)NHCO-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh  
 20 (from Step 5 above, 0.5 g, 0.52 mmol) in MeOH (5 mL)  
 was treated with Pyridine·HCl (catalytic). The  
 resulting mixture was stirred at 65°C for 6 hours.  
 Concentrated in vacuo to obtain an oil which was  
 purified by by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH  
 25 eluent) to give (4-EtO-Ph)NHCO-D-His-Tyr(OBn)-  
 CONH(CH<sub>2</sub>)<sub>3</sub>OPh, mp 185-187°C; ES-MS 705 (m+1).

## EXAMPLE 26

(S,R)-N-{2-(4-Benzoyloxy-phenyl)-1-[3-(2-methoxy-  
 30 phenyl)-propylcarbamoyl]-ethyl}-3-(3H-imidazol-4-yl)-  
2-phenylacetyl-amino-propionamide {PhCH<sub>2</sub>CO-D-His-  
Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh)}

Step 1. Boc-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh)

According to Example 25, Step 1, by substituting  
 35 3-(2-methoxyphenyl) propyl amine for 2-(phenylmethoxy)-

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ethylamine, the title compound was obtained as a white solid, mp 125-126.5°C.

Step 2. Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh)

5        According to Example 25, Step 2, by substituting Boc-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh) for Boc-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh, the title compound was obtained as a white solid; CI-MS 419 (m+1).

10      Step 3. Fmoc-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh)

         According to Example 25, Step 3, by substituting Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh) for Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh, the title compound was obtained as a foam; ES-MS 1020 (m).

15

Step 4. D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh)

         According to Example 25, Step 4, by substituting Fmoc-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh) for Fmoc-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh, the title compound was  
20      obtained as a white foam; ES-MS 798 (m+1).

Step 5. PhCH<sub>2</sub>CO-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh)

         To a solution of D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh) (0.4 g, 0.5 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL)  
25      was added N-methyl morpholine (0.05 g, 0.5 mmol) followed by phenyl acetyl chloride (0.08 g, 0.5 mmol). The resulting mixture was stirred 2 hours at room temperature. Diluted with DCM and washed with saturated aqueous NaHCO<sub>3</sub>, saturated aqueous NaCl, and  
30      dried (MgSO<sub>4</sub>). Purified by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent). The title compound was obtained as a foam; FAB-MS 916 (m).

Step 6. PhCH<sub>2</sub>CO-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh)

35      According to Example 25, Step 6, by substituting PhCH<sub>2</sub>CO-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>(2-MeOPh) for

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(4-EtOPh)NHCO-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh, the title compound was obtained as a white foam; ES-MS 674 (m+1).

5

## EXAMPLE 27

(S,R)-N-[2-(4-Benzoyloxy-phenyl)-1-phenethylcarbamoyl-ethyl]-3-(3H-imidazol-4-yl)-2-[3-(4-phenoxy-phenyl)-ureido]-propionamide {(4-PhOPh)NHCO-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph}

10 Step 1. Boc-Tyr(OBn)CONH(CH<sub>2</sub>)<sub>2</sub>Ph

According to Example 25, Step 1, by substituting phenethylamine for 2-(phenylmethoxy)ethylamine, the title compound was obtained as a white solid; CI-MS 475 (m+1).

15

Step 2. Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph

According to Example 25, Step 2, by substituting BocTyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph for BocTyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh, the title compound was obtained as a white solid;

20 CI-MS 375 (m+1).

Step 3. Fmoc-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph

According to Example 25, Step 3, by substituting Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph for Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh, the title compound was obtained as a foam; ES-MS 977 (m+1).

25

Step 4. Fmoc-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph

Fmoc-D-His(Tr)-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph (1.2 g, 1.6 mmol) in MeOH (5 mL) was treated with Pyridine·HCl (catalytic). The resulting mixture was stirred at 65°C overnight and concentrated in vacuo to obtain an oil which was purified by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent) to give a white solid; ES-MS 734 (m+1).

35

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Step 5. (4-PhOPh)NHCO-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph

Fmoc-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph (0.6 g, 0.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was treated with piperidine (0.14 g, 1.6 mmol). The resulting mixture was stirred 2 hours before concentrating in vacuo and purifying the resulting by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent) to give D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph. The foam was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and treated with 4-phenoxyphenyl isocyanate (0.05 g, 0.23 mmol). The resulting mixture was stirred 1 hour at room temperature, concentrated in vacuo, and purified the resulting oil by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent) to obtain (4-PhOPh)NHCO-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph as a foam; ES-MS 723 (m+1).

## EXAMPLE 28

(S,R)-N-[1-(4-Benzoyloxy-benzyl)-2-(4-benzyl-piperazin-1-yl)-2-oxo-ethyl]-3-(3H-imidazol-4-yl)-2-(toluene-4-sulfonylamino)-propionamide {(4-MePh)SO<sub>2</sub>-D-His-Tyr(OBn)-CO(4-Bn-piperazin-1-yl)·HCl}

Step 1. Boc-Tyr(OBn)-CO(4-Bn-piperazin-1-yl)

According to Example 25, Step 1, by substituting 1-benzylpiperazine for 2-(phenylmethoxy)ethylamine, the title compound was obtained as a white solid; CI-MS 530 (m+1).

Step 2. Tyr(OBn)-CO(4-Bn-piperazin-1-yl)

According to Example 25, Step 2, by substituting Boc-Tyr(OBn)-CO(4-Bn-piperazin-1-yl) for Boc-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh, the title compound was obtained as a white solid; CI-MS 430 (m+1).

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Step 3. Fmoc-D-His(Tr)-Tyr(OBn)-CO(4-Bn-piperazin-1-yl)

According to Example 25, Step 3, by substituting Tyr(OBn)-CO(4-Bn-piperazin-1-yl) for Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh, the title compound was obtained as a foam; ES-MS 1032 (m+1).

Step 4. (4-MePh)SO<sub>2</sub>-D-His(Tr)-Tyr(OBn)-CO(4-Bn-piperazin-1-yl)

Fmoc-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh, (0.7 g, 0.69 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) was treated with piperidine (0.14 g, 1.6 mmol). The resulting mixture was stirred 2 hours before concentrating in vacuo and purifying the resulting oil by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent) to give D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>2</sub>Ph. The foam was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and treated with pyridine (0.05 g, 0.63 mmol), followed by 4-toluenesulfonyl chloride (0.12 g, 0.63 mmol). The resulting mixture was stirred 3 hours at room temperature, concentrated in vacuo, and purified the resulting oil by flash chromatography (SiO<sub>2</sub>, CHCl<sub>3</sub>:MeOH eluent) to obtain (4-MePh)SO<sub>2</sub>-D-His(Tr)-Tyr(OBn)-CO(4-Bn-piperazin-1-yl); ES-MS 963 (m).

Step 5. (4-MePh)SO<sub>2</sub>-D-His-Tyr(OBn)-CO(4-Bn-piperazin-1-yl)·HCl

(4-MePh)SO<sub>2</sub>-D-His(Tr)-Tyr(OBn)-CO(4-Bn-piperazin-1-yl) (0.21 g, 0.22 mmol) was treated with 80% aqueous HCl (3 mL) and heated to 80°C for 5 minutes. The mixture was cooled and diluted with water (5 mL). The solid was filtered off, and the supernate was concentrated in vacuo to provide an oil. The oil was dissolved in water (15 mL), frozen, and lyophilized to provide (4-MePh)SO<sub>2</sub>-D-His-Tyr(OBn)-CO(4-Bn-piperazin-1-yl)·HCl; ES-MS 720 (m).



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## EXAMPLE 29

N<sub>α</sub>-[N-[(Phenylmethoxy)carbonyl]-L-histidyl]-O-(phenylmethyl)-N-methyl-N-[2-(phenylmethoxy)ethyl]-L-tyrosinamide {Cbz-His-Tyr(OBn)-CON(Me)CH<sub>2</sub>CH<sub>2</sub>OBn}

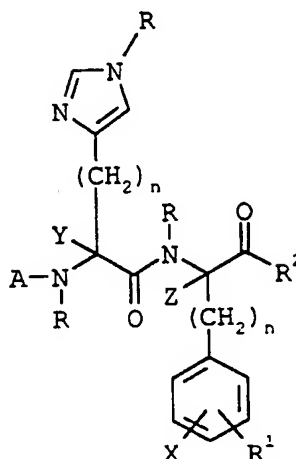
5           According to Example 19, Step 3, by substituting N-methyl-N-[2-(phenylmethoxy)ethyl]amine for 2-(phenylmethoxy)ethylamine, the title compound was prepared; FAB-MS 690 (m+1).

10           The present invention may be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments are to be considered in all respects only as illustrative and not restrictive. The scope of the  
15           invention is, therefore, indicated by the appended claims rather than by the foregoing description. All changes which come within the meaning and range of equivalency of the claims are to be embraced within their scope.

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## CLAIMS

1. A compound of the Formula I:



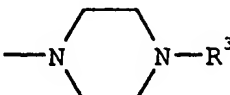
wherein:

- 15       $n = 1 \text{ or } 2$ ;  
           $A = \text{COR}^3, \text{CO}_2\text{R}^3, \text{CONHR}^3, \text{CSR}^3, \text{C(S)OR}^3, \text{C(S)NHR}^3,$   
           $\text{CF}_3\text{SO}_2, \text{aryl-SO}_2, \text{ or alkyl-SO}_2, \text{ wherein } \text{R}^3 \text{ is}$   
           $\text{alkyl, } (\text{CH}_2)_m\text{-cycloalkyl, } (\text{CH}_2)_m\text{-aryl,}$   
           $(\text{CH}_2)_m\text{-heteroaryl, or } (\text{CH}_2)_m\text{O-alkyl, and } m =$   
 20       $0, 1, 2, \text{ or } 3$ ;  
           $R = \text{independently H or Me};$   
           $Y = \text{independently H or Me};$   
           $Z = \text{independently H or Me};$   
           $R^1 = \text{H, CO-aryl, } (\text{CH}_2)_m\text{-aryl, O(CH}_2)_m\text{-cycloalkyl,}$   
 25       $\text{O(CH}_2)_m\text{-aryl, or O(CH}_2)_m\text{-heteroaryl wherein } m$   
           $\text{is as defined above and } R^1 \text{ is located at}$   
           $\text{either the meta or para position};$   
           $X = \text{one to four substituents, including H, alkyl,}$   
           $\text{CF}_3, \text{F, Cl, Br, I, HO, MeO, NO}_2, \text{NH}_2, \text{N(Me)}_2,$   
 30       $\text{OPO}_3\text{H}_2, \text{ or CH}_2\text{PO}_3\text{H}_2; \text{ and}$   
           $R^2 = \text{NR(CH}_2)_n\text{CO}_2\text{R}^3, \text{NR(CH}_2)_n\text{CONHR}^3, \text{NR(CH}_2)_n\text{R}^3,$   
           $\text{NR(CH}_2)_{n+1}\text{OR}^4, \text{NR(CH}_2)_{n+1}\text{SR}^4,$

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NRCH(COR<sup>5</sup>)(CH<sub>2</sub>)<sub>n</sub>-heteroaryl,  
 NRCH(COR<sup>5</sup>)(CH<sub>2</sub>)<sub>n</sub>OR<sup>3</sup>, NRCH(COR<sup>5</sup>)(CH<sub>2</sub>)<sub>n</sub>SR<sup>3</sup>,

35

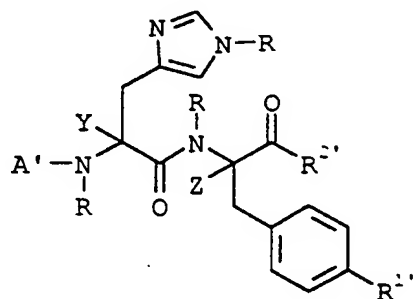
or  wherein R, R<sup>3</sup>, and n are

as defined above, R<sup>4</sup> = H or R<sup>3</sup>, and R<sup>5</sup> = OH,  
 NH<sub>2</sub>, OR<sup>3</sup>, or NHR<sup>3</sup>; an optical isomer,  
 diastereomer, or a pharmaceutically  
 acceptable salt thereof.

40

2. A compound according to Claim 1 which is a  
 compound of Formula II:

5



II

10

wherein:

A' = CO<sub>2</sub>R<sup>3</sup>, CONHR<sup>3</sup>, C(S)NHR<sup>3</sup>, or aryl-SO<sub>2</sub>, wherein  
 R<sup>3</sup> is alkyl, (CH<sub>2</sub>)<sub>m</sub>-cycloalkyl, (CH<sub>2</sub>)<sub>m</sub>-aryl,  
 (CH<sub>2</sub>)<sub>m</sub>-heteroaryl, and m = 0, 1, 2 or 3;

15

R = independently H or Me;

Y = independently H or Me;

Z = independently H or Me;

R<sup>1'</sup> = (CH<sub>2</sub>)<sub>m</sub>-aryl, O(CH<sub>2</sub>)<sub>m</sub>-aryl, OPO<sub>3</sub>H<sub>2</sub>, or  
 CH<sub>2</sub>PO<sub>3</sub>H<sub>2</sub>, wherein m is as defined above;

20

R<sup>2'</sup> = NR(CH<sub>2</sub>)<sub>2</sub>OR<sup>4</sup>, NR(CH<sub>2</sub>)<sub>2</sub>SR<sup>4</sup>, NRCH(COR<sup>5</sup>)CH<sub>2</sub>OR<sup>3</sup>, or  
 NRCH(COR<sup>5</sup>)CH<sub>2</sub>SR<sup>3</sup>, wherein R<sup>3</sup> and n are as  
 defined above, R<sup>4</sup> = H or R<sup>3</sup>, and R<sup>5</sup> = OH,  
 NH<sub>2</sub>, OR<sup>3</sup>, or NHR<sup>3</sup>; an optical isomer,  
 diastereomer, or a pharmaceutically  
 acceptable salt thereof.

25

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3. A compound according to Claim 1 wherein A is  $\text{CO}_2\text{R}^3$  or  $\text{CONHR}^3$ .
4. A compound according to Claim 1 wherein at least one of Y and Z is Me.
5. A compound according to Claim 1 wherein  $\text{R}^2$  is  $\text{NH}(\text{CH}_2)_2\text{OR}^4$  or  $\text{NHCH}(\text{COR}^5)\text{CH}_2\text{OR}^3$ .
6. A compound according to Claim 1 wherein A is  $\text{NHCONHR}^3$ ,  $\text{R}^2$  is  $(\text{CH}_2)_2\text{OR}^4$ , and at least one of Y and Z is Me.
7. A compound according to Claim 1 selected from the group consisting of:
  - Cbz-His-Tyr(OBn)-Ser(OBn)- $\text{CO}_2\text{Me}$ ;
  - Cbz-His-Tyr(OBn)-Ser(OBn)- $\text{CONH}_2$ ;
  - 5 Cbz-His-Tyr(OBn)-Ser(OBn)-CONH<sub>Et</sub>; and
  - Cbz-His-Tyr(OBn)-Ser(OBn).
8. A compound according to Claim 1 selected from the group consisting of:
  - Cbz-His-Tyr(OBn)-D-Ser(OBn)- $\text{CO}_2\text{Me}$ ;
  - Cbz-D-His-Tyr(OBn)-Ser(OBn)- $\text{CONH}_2$ ;
  - 5 Cbz-D-His-Tyr(OBn)-Ser(OBn)-CONH<sub>Et</sub>;
  - Cbz-D-His-Tyr(OBn)-Ser(OBn)- $\text{CO}_2\text{Me}$ ; and
  - Cbz-D-His-Tyr(OBn)-Ser(OBn).
9. A compound according to claim 1 selected from the group consisting of:
  - Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn)- $\text{CO}_2\text{Me}$ ;
  - Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn)- $\text{CONH}_2$ ;
  - 5 Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn)-CONH<sub>Et</sub>;
  - Cbz-His(1-Me)-Tyr(OBn)-Ser(OBn);
  - Cbz-D-His(1-Me)-Tyr(OBn)-Ser(OBn)- $\text{CO}_2\text{Me}$ ;
  - Cbz-D-His(1-Me)-Tyr(OBn)-Ser(OBn)- $\text{CONH}_2$ ;

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10                    Cbz-D-His(1-Me)-Tyr(Obn)-Ser(Obn)-CONH<sub>2</sub>; and  
                     Cbz-D-His(1-Me)-Tyr(Obn)-Ser(Obn).

10. A compound according to claim 1 selected from the group consisting of:

5      Cbz-His- ( $\alpha$ -Me) Tyr (OBn) - Ser (OBn) - CO<sub>2</sub>Me;  
Cbz-His- ( $\alpha$ -Me) Tyr (OBn) - Ser (OBn) - CONH<sub>2</sub>;  
Cbz-His- ( $\alpha$ -Me) Tyr (OBn) - Ser (OBn) - CONHEt;  
Cbz-His- ( $\alpha$ -Me) Tyr (OBn) - Ser (OBn) ;  
Cbz-His-D- ( $\alpha$ -Me) Tyr (OBn) - Ser (OBn) - CO<sub>2</sub>Me;  
Cbz-His-D- ( $\alpha$ -Me) Tyr (OBn) - Ser (OBn) - CONH<sub>2</sub>;  
Cbz-His-D- ( $\alpha$ -Me) Tyr (OBn) - Ser (OBn) - CONHEt; and  
10      Cbz-His-D- ( $\alpha$ -Me) Tyr (OBn) - Ser (OBn) .

11. A compound according to Claim 1 selected from the group consisting of:

5                    Cbz-D-His-homoTyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;  
                     Cbz-His-Phe(4-Ph)-Ser(OBn)-CO<sub>2</sub>Me;  
                     Cbz-D-His-Phe(4-Ph)-Ser(OBn)-CO<sub>2</sub>Me;  
                     Cbz-His-Tyr(OBn)-Pyr-CO<sub>2</sub>Me; and  
                     Cbz-D-His-Tyr(OBn)-Pyr-CO<sub>2</sub>Me.

12. A compound according to Claim 1 selected from the group consisting of:

5      Cbz-His-Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>OBn;  
Cbz-D-His-Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>OBn;  
Cbz-His- (N-Me) Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>OBn;  
Cbz-D-His- (N-Me) Tyr (OBn) - CONHCH<sub>2</sub>CH<sub>2</sub>OBn;  
Cbz-His-Tyr (OBn) - CONH(CH<sub>2</sub>)<sub>2</sub>Ph; and  
Cbz-D-His-Tyr (OBn) - CONH(CH<sub>2</sub>)<sub>2</sub>Ph.

13. A compound according to Claim 1 selected from the group consisting of:

Cbz-His-Tyr(OBn)-Gly-CO<sub>2</sub>Bn;  
Cbz-D-His-Tyr(OBn)-Gly-CO<sub>2</sub>Bn;

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- 5                    Cbz-His-Tyr(OBn)-Gly-CONHBn; and  
                    Cbz-D-His-Tyr(OBn)-Gly-CONHBn.
14.    A compound according to Claim 1 selected from the  
         group consisting of:  
         BnNHCO-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;  
         BnNHCO-His-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;  
5           BnNHCO-His-Tyr(OBn)-Ser(OBn)-CONHEt;  
         BnNHCO-His-Tyr(OBn)-Ser(OBn);  
         BnNHCO-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>OBn; and  
         BnNHCO-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OPh.
15.    A compound according to Claim 1 selected from the  
         group consisting of:  
         BnNHCO-D-His-Tyr(OBn)-Ser(OBn)-CO<sub>2</sub>Me;  
         BnNHCO-D-His-Tyr(OBn)-Ser(OBn)-CONH<sub>2</sub>;  
5           BnNHCO-D-His-Tyr(OBn)-Ser(OBn)-CONHEt;  
         BnNHCO-D-His-Tyr(OBn)-Ser(OBn);  
         BnNHCO-D-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>OBn; and  
         BnNHCO-D-His-Tyr(OBn)-CONHCH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>OPh.
16.    A compound according to Claim 1 selected from the  
         group consisting of:  
         Cbz-His-Tyr(OBn)-CON(Me)CH<sub>2</sub>CH<sub>2</sub>OBn;  
         (4-EtOPh)NHCO-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>OPh;  
5           PhCH<sub>2</sub>CO-D-His-Tyr(OBn)-CONH(CH<sub>2</sub>)<sub>3</sub>-(2-MeOPh);  
         (4-PhOPh)NHCO-D-His-Tyr(OBn)-COHN(CH<sub>2</sub>)<sub>2</sub>Ph;  
         and  
         (4-MePh)SO<sub>2</sub>-D-His-Tyr(OBn)-CO(4-Bn-  
         piperazin-1-yl).
17.    A method of treating tissue proliferative diseases  
         comprising administering to a host suffering  
         therefrom a therapeutically effective amount of a  
         compound according to Claim 1 in unit dosage form.

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18. A pharmaceutical composition adapted for administration as an antiproliferative agent comprising a therapeutically effective amount of a compound according to Claim 1 in admixture with a pharmaceutically acceptable excipient, diluent or carrier.
19. A method of treating cancer comprising administering to a host suffering therefrom a therapeutically effective amount of a compound according to Claim 1 in unit dosage form.
20. A pharmaceutical composition adapted for administration as an anticancer agent comprising a therapeutically effective amount of a compound according to Claim 1 in admixture with a pharmaceutically acceptable excipient, diluent or carrier.
21. A method of treating restenosis comprising administering to a host suffering therefrom a therapeutically effective amount of a compound according to Claim 1 in unit dosage form.
22. A pharmaceutical composition adapted for administration as a restenosis inhibiting agent comprising a therapeutically effective amount of a compound according to Claim 1 in admixture with a pharmaceutically acceptable excipient, diluent, or carrier.
23. A process for the preparation of organic compounds according to Claim 1, or a pharmaceutically acceptable salt thereof, comprising the steps of employing solid phase support technology and sequentially coupling building blocks utilizing a

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10 solid phase peptide synthesizer, cleaving the organic compound from the solid phase support and subsequently optionally modifying the C-terminus of the organic compound in solution phase to afford a compound or a pharmaceutically acceptable salt thereof of Formula I.

24. A process for the preparation of compounds according to Claim 1, or a pharmaceutically acceptable salt thereof, comprising the steps of employing solution phase technology and  
5 sequentially coupling building blocks to afford a compound or a pharmaceutically acceptable salt thereof of Formula I.

25. A process for the preparation of compounds according to Claim 1, or a pharmaceutically acceptable salt thereof, comprising simultaneous synthesis of compounds of Formula I in a multiple  
5 simultaneous synthesis apparatus, using a D- or L-histidine containing dipeptide derivative that is supported on 2-chlorotrityl resin with sequential deprotection and acylation of the N-terminus followed by sequential deprotection of the carboxy  
10 terminus, carboxyl activation and condensation with a series of amines, followed by cleavage from the solid support.



## INTERNATIONAL SEARCH REPORT

Intern al Application No

PCT/US 94/11553

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K5/078 C07K5/097 A61K38/05 A61K38/06

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BIOCHEMISTRY., vol.8, no.2, February 1969, EASTON, PA US pages 575 - 585 T.R.HOLLANDS ET AL 'Action of pepsin on cationic synthetic substrates' see page 577, column 1; table II ---	1,3,24
X	BIOCHEMISTRY., vol.9, no.5, 3 March 1970, EASTON, PA US pages 1154 - 1162 K.MEDZIHRADSKY ET AL 'Effect of secondary enzyme-substrate interactions on the cleavage of synthetic peptides by pepsin' see page 1157, column 1 --- -/--	1,3,24



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

31 January 1995

Date of mailing of the international search report

15 -02- 1995

Name and mailing address of the ISA

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Groenendijk, M

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/US 94/11553

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 523 873 (MERCK & CO) 20 January 1993 cited in the application see the whole document -----	1-25

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 94/11553

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark : Although claims 17, 19 and 21 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

information on patent family members

Internal Application No

PCT/US 94/11553

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0523873	20-01-93	CA-A- 2072033 JP-A- 6157589	29-12-92 03-06-94
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